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THE INFLUENCE OF PROLONGED COLD EXPOSURE ON LACTATION AND GROWTH IN SHEEP

by

© Gwen Elizabeth McBride

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
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Supervisor

....

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Date..December 7, 1982......

Abstract

Experiments were performed to determine the effect of prolonged cold exposure on the lactational performance, feed digestibility, organ development and mammary blood flow of lactating ewes, to determine the degree of diurnal variation and to examine the influence of acute stress on mammary blood flow and to assess the growth, morphological and digestive responses of young lambs to a cold environment.

Exposure of lactating ewes to 0°C for 24 h resulted in a 20 % increase in heat production, which rose to a level 55 % higher than thermoneutral values after 41 days of treatment. Digestibility of a 75 % barley-25 % alfalfa diet, measured after 50 days of cold exposure, was reduced by 3.8 % ($P<0.05$). Plasma noradrenaline and triiodothyronine levels were elevated ($P<0.05$) in the cold-exposed ewes and plasma adrenaline, thyroxine and glucose levels also tended to be increased. Chronic cold exposure had no influence on plasma levels of insulin.

Despite the decreased feed digestibility and the increased metabolic demands of the ewes, milk production was not significantly altered by cold treatment. Milk concentrations of fat and protein tended to be higher in the cold-exposed ewes but lactose concentration remained unchanged. Milk fat was characterized by a reduced ($P<0.01$) proportion of short chain fatty acids as a result of cold exposure.

Cold stress resulted in a significant degree of adrenal gland hypertrophy ($P<0.05$) and a tendency of increased thyroid weight but had no influence on the weight of the gastro-intestinal tract.

After 8 wk of cold treatment mammary gland weight/unit bodyweight was reduced ($P<0.05$). Analysis of the tissue composition of the right half of the udder revealed a tendency of decreased secretory tissue mass as a result of cold exposure. Total mammary blood flow ($ml.min^{-1}$, $ml.100g^{-1}.min^{-1}$) measured with radionuclide labelled microspheres also appeared to be depressed as a consequence of cold exposure. As a proportion of total mammary blood flow, secretory tissue blood flow was reduced by approximately the same amount as connective tissue blood flow was increased in the cold-exposed ewes. Suckling resulted in a tendency of reduced mammary blood flow in both the control and cold-exposed ewes but had no influence on blood flow distribution within the mammary gland. Blood flow to the popliteal adipose depot of the hindlimb increased over three-fold as a result of cold exposure. Blood flow to selected muscles of the hindleg was slightly increased although blood flow to the skin of the leg was reduced as a consequence of cold exposure.

Continuous recording of blood flow through the right mammary artery with an electromagnetic blood flow probe revealed that changes in posture, feeding, minor stresses and suckling caused blood flow to decrease. During acute

cold exposure, mammary blood flow and udder surface temperatures fell markedly when the ewe was standing but lying down effectively insulated the mammary gland from the cold.

Young suckling lambs housed at 0°C had a slightly depressed rate of growth despite nutrient intakes similar to the lambs housed at a neutral temperature. Weight gain over a short period following weaning also tended to be lower as a consequence of cold exposure. Cold-exposed lambs were characterized by a tendency of reduced ear size and decreased leg bone length, particularly of the more distal bones of the hindlimb. Although the dry matter and nitrogen digestibilities of a concentrate ration fed to the weaned lambs were not affected by cold exposure, because of a relative increase in urinary nitrogen loss, nitrogen retention was reduced ($P<0.05$). Organ weights measured after 11 wk of differential temperature exposure were similar for both groups although the increased thyroid and abomasal weights of the cold-exposed lambs approached statistical significance ($P<0.10$).

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I wish to dedicate this thesis to my husband Brian to whom I am indebted for his unfailing support, encouragement and advice given throughout this entire project.

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I. Introduction

Exposure to a cold environment elicits certain behavioral, physiological and biochemical changes designed to enable the animal to maintain a state of homeothermy. In general, the influence of a low ambient temperature acts through alterations of the neural, neuro-endocrine and endocrine control systems of the animal, resulting in:

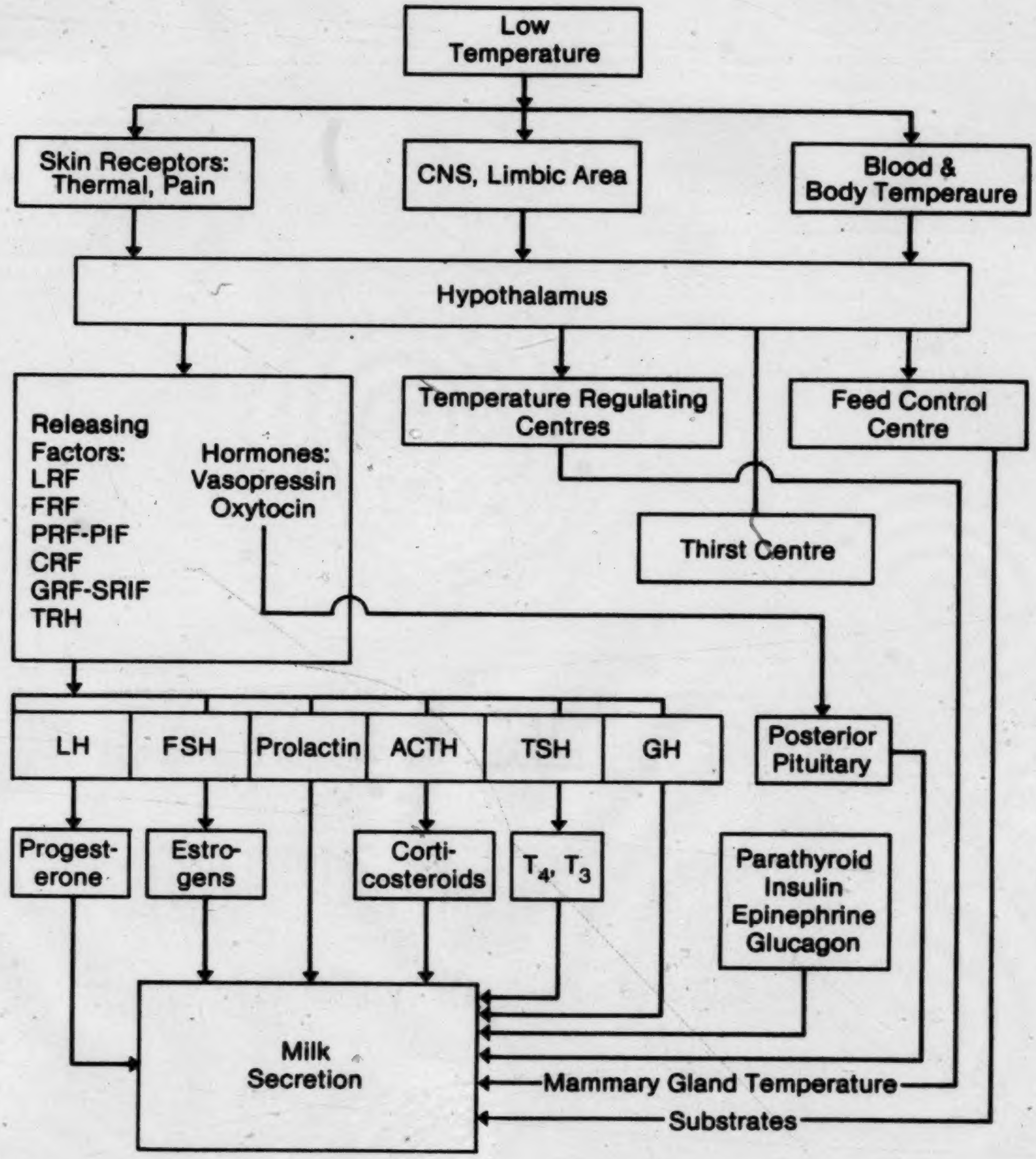
1. changes in behavior (ie. posture and activity) (Gonyou et al. 1979) that help to minimize heat loss to the environment.
2. peripheral vasoconstriction (Alexander 1979; Thauer 1965; Thompson 1977) which increases the tissue insulation of the animal, thus reducing convective and conductive heat losses.
3. increased blood flow to thermogenic tissues (ie. skeletal muscle, brown adipose tissue) to support both shivering and non-shivering forms of thermogenesis (Thompson 1977).
4. increased heart rate and cardiac output (Hales et al. 1976; Thompson et al. 1975) to help support an increased metabolic rate.
5. a greater metabolic activity of the thyroid gland and sympathetico-adrenal medullary system which has been suggested to be important in co-ordinating and regulating the animal's response to a cold environment (Gale 1973).

The influence of these cold-induced events on the process of milk formation has received little attention. Possible mechanisms by which temperature and other biometeorlogical factors may affect milk production have been suggested by Johnson (1976) and are presented in Figure I.1. Since milk formation depends upon the supply and uptake of nutrients by the mammary gland, a low ambient temperature potentially could restrict the animal's capacity for milk production by:

1. restricting nutrient supply through:
    a. a reduction of feed digestibility.
    b. a diversion of nutrients away from milk production to meet the increased maintenance energy demands of the lactating animal.
    c. an alteration of mammary blood flow.
2. altering the endocrine balance necessary for milk secretion.
3. decreasing the rate of mammary metabolism because of reduced tissue temperature.

What has not been considered by Johnson (1976) or other researchers (Clarke et al. 1976; Faulkner et al. 1980; Thompson and Thomson 1977; Thomson et al. 1979) is the influence of the suckling young on the capacity of a cold-stressed animal to produce milk. In a situation where both the lactating animal and the suckling offspring are housed in a cold environment, the increased energy demands of the offspring may result in an increased appetite drive,

Figure I.1 Influence of Cold on the Neural, Neuro-endocrine and Endocrine Systems and the Resultant Effects on Milk Secretion.


Low Temperature
Skin Receptors: Thermal, Pain
CNS, Limbic Area
Blood & Body Temperaure
Hypothalamus
Releasing Factors:
LRF
FRF
PRF-PIF
CRF
GRF-SRIF
TRH
Hormones:
Vasopressin
Oxytocin
Temperature Regulating Centres
Feed Control Centre
Thirst Centre
LH
FSH
Prolactin
ACTH
TSH
GH
Posterior Pituitary
Progesterone
Estrogens
Corticosteroids
T_4, T_3
Parathyroid
Insulin
Epinephrine
Glucagon
Milk Secretion
Mammary Gland Temperature
Substrates

Adapted from
Johnson (1976)

which in turn may stimulate milk production. Since, in the ewe, it has been suggested that maximum lactational performance is not achieved when only one lamb is being nursed, a cold-exposed ewe nursing a single lamb may have the genetic potential to increase milk production in response to an increased appetite drive of the lamb.

The principal purpose of the experiments presented in the following chapters was to investigate the effect of a cold environment on the lactating ewe nursing a single lamb. In addition to examining the lactational performance of the ewe, other factors such as energy and endocrine balance, feed digestibility, substrate availability and mammary blood flow were considered because of their influence on the process of milk formation. As well, the digestibility and nitrogen retention of a concentrate diet fed to the lambs after weaning were examined to determine if similar digestive responses to cold occur in young growing lambs as in mature sheep. Finally, the morphology of young growing lambs housed in a cold or thermoneutral environment were compared to investigate the possibility of a cold-induced adaptation in growth which may be of benefit to the thermal balance of the cold-exposed lamb.

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II. Section I

The Effects of Prolonged Cold Exposure During Lactation on the Feed Digestion, Endocrine Balance, Milk Production and Milk Composition of the Ewe

A. Abstract

The lactational performances of ewes chronically exposed to a thermoneutral (21±1 °C) or cold (0±1 °C) environment were compared in two experiments. Evidence of cold stress included a depression in feed digestibility ($P<0.05$), and an immediate increase in heat production ($P<0.05$) and plasma concentrations of noradrenaline ($P<0.05$), adrenaline ($P>0.10$) and glucose ($P<0.10$). Acclimation to a low temperature was reflected by a further rise in heat production ($P<0.05$) and plasma T_3 and T_4 levels ($P<0.05$), but a gradual reduction of plasma noradrenaline ($P<0.05$) and adrenaline ($P>0.10$) concentrations. Plasma insulin levels were not affected by thermal treatment. Cold exposure tended to result in an increase in percent milk fat and protein, however lactose concentration remained unchanged. Milk obtained from the cold-exposed ewes was characterized by a relative decrease in short chain fatty acid secretion ($P<0.01$). Although milk composition was affected by cold stress, daily milk production was not significantly altered; as a consequence, total energy lost in the milk tended to be slightly higher for the cold-stressed ewes. The increased energy lost as heat and in

the milk during cold exposure resulted in a net energy deficit throughout most of the experimental period. Prolonged cold exposure (8 wk) did not affect gastrointestinal tract, lung or kidney weights (P>0.10), although mammary gland weight, expressed per unit of body weight, was reduced (P<0.05). Adrenal gland weight was significantly greater in the cold-exposed ewes (P<0.05) and thyroid gland weight also tended to be higher as a result of cold exposure (P>0.10). Efficiency of milk secretion calculated in terms of g milk/g mammary or secretory tissue and mammary blood flow:milk yield reflected an increase in milk production efficiency as a result of prolonged cold exposure.

B. Introduction

At ambient temperatures below the lower limit of the thermoneutral zone, sheep lose more energy to the environment (Graham et al. 1959) and frequently, digestion is impaired (Christopherson 1976; Kennedy et al. 1976,1982; Westra and Christopherson 1976); consequently, if the energy intake of a lactating ewe is fixed during cold exposure, less energy is available to meet the maintenance and lactational demands of the animal. Potentially then, the quantity and nature of the nutrients secreted in the milk and available to the suckling young may be affected. Cold exposure may also directly limit the synthetic capacity of the mammary gland by depressing the rate of mammary metabolism, as suggested by Johnson (1976), or may act indirectly, by affecting the udder's blood supply (Thompson and Thomson 1977; Thomson et al. 1979; Thompson 1980). As well, changes in endocrine metabolism induced by cold exposure (see reviews by Gale 1973; Thompson 1977; Alexander 1979) might significantly alter the metabolism of the mammary gland (Faulkner et al. 1980; Robertshaw 1981). These developments could serve to further limit the milk producing capacity of the cold-stressed ewe.

On the other hand, plasma concentrations of metabolites that can serve as mammary substrates, such as glucose (Alexander et al. 1968; McKay et al. 1974), free fatty acids (Alexander et al. 1968; Thompson et al. 1975) and glycerol (Thompson et al. 1975), rise when sheep are exposed to a cold environment. These metabolites are used as fuel by shivering muscle (Bell et al. 1975; Bell and Thompson 1979) but a change in their plasma concentrations may also affect the quantity and nature of the substrates taken up by the mammary gland (Clarke et al. 1976; Thomson et al. 1979; Faulkner et al. 1980). Cold stress may also induce an increased appetite drive in the young suckling lambs, as has been shown to occur in cold-stressed mature sheep (Baile and Forbes 1974). Increased suckling by the lambs may in turn stimulate mammary metabolism. The net effect of these many factors on the milk production of the cold-stressed lactating ewe is difficult to predict.

The present experiments were designed to examine the influence of prolonged cold exposure during lactation on the digestive responses, metabolic rate, plasma concentrations of hormones and of glucose, and milk production and composition of the ewe.

C. Materials and Methods

Experiment I

Animals

Five months prior to the start of the experiment estrus was synchronized using a hormone treatment (progesterone impregnated vaginal sponge, 500 iu PMSG injection) in a group of 16 Suffolk-cross 2 year-old ewes. Seven to 10 days pre-partum the ewes were introduced to the experimental ration, consisting of a pelleted 75% barley-25% alfalfa meal mixture (Table II.1). One to 3 days post-partum, 8 ewes, 4 nursing single and 4 nursing twin lambs, were selected; ewes nursing two offspring were reduced to a single lamb at this time. According to bodyweight and sex of lamb, type of birth (single, twin) and ewe bodyweight, the ewes were assigned for an 8 wk period to one of two balanced treatment groups:

1. Control (CON): housed at a thermoneutral temperature (21±1 °C).
2. Cold-Exposed (CE): housed at a temperature of 0±1 °C.

Each ewe-lamb pair was confined to a 1.2x1.2 m metabolic crate within a continuously-lit animal room or temperature-controlled chamber, initially maintained at 21±1 °C. During a 5-7 day adaptation period, the animals were accustomed to the experimental procedures and equipment. Additionally, the ewes received intramuscular

Table II.1 Diet Composition, Experiment I

Ingredient:	% as fed
Rolled Barley	73.0
Alfalfa Meal	24.0
Cobalt-Iodized Salt	1.0
Dicalcium Phosphate	1.0
Vitamin ADE Premix	1.0

injections (mg) of retinol 75, cholecalciferol 0.94 and α-tocopherol 16 and were shorn to a fleece depth of approximately 1 cm. To maintain a relatively constant level of insulation, the ewes were reshorn at 2 wk intervals. During the adaptation and experimental periods, feed was offered twice daily to each ewe (0800 & 1600 h) at a level to maintain bodyweight. Based on weekly weight measurements, it was necessary to feed the CON ewes at a level of 100 $g.kg^{-0.75}.day^{-1}$ while the CE ewes received 112 $g.kg^{-0.75}.day^{-1}$. Free access to water and cobalt-iodized salt was also provided.

Measurements

A summary of the manipulations made during this experiment is presented in Figure II.1.

Heat Production

The heat production of the ewes was monitored during wk 1, 4 and 7 of the experimental period, corresponding respectively to wk 2, 5 and 8 of lactation. The first set of measurements were performed after the CE ewes had been exposed for 24 h to 0 °C. Estimation of heat production was based on the measurements of respired gas, VO_2 and VCO_2, over a 24 h period using an open circuit respiratory pattern analyzer (Young et al. 1975) connected to a respiratory hood (75x70x45 cm), through which air was drawn at a rate of 70 - 75 $l.min^{-1}$, corrected for standard temperature and pressure. This apparatus allowed normal

Figure II.1 Sequence of Animal Manipulations and Measurements, Experiment I.

Length on Trial		Stage of Lactation		Measurement		
Week	Days	Week	Days	Weight	Metabolic Rate	Milk Production
1	1	1	5	x		
	2		6			
	3		7			
	4	2	8			
	5		9			
	6		10		x	
	7		11		x	x
2	8		12	x	x	
	9		13		x	
	10		14		x	
	11	3	15			
	12		16			
	13		17			x
	14		18			
3	15		19			
	16		20	x		
	17		21			
	18	4	22			
	19		23			
	20		24			x
	21		25			
4	22		26	x		
	23		27			
	24		28			
	25	5	29			
	26		30			
	27		31		x	x
	28		32		x	
5	29		33	x	x	
	30		34		x	
	31		35		x	
	32	6	36			
	33		37			
	34		38			x
	35		39			
6	36		40	x		
	37		41			
	38		42			
	39	7	43			
	40		44			
	41		45			x
	42		46			
7	43		47			
	44		48	x		
	45		49			
	46	8	50			
	47		51		x	
	48		52		x	x
	49		53		x	
8	50		54	x	x	
	51		55		x	
	52		56			
	53	9	57			
	54		58			
	55		59			
	56		60			
9	57		61	x		

Adjustment Period

Differential Treatment Period

access of the ewe to feed and water but did not restrict lying, standing or nursing activities. Through a system of valves that automatically alternated the source of respired gas being analyzed every 15 min, two animals could be simultaneously monitored over each 24 h period.

Milk Production and Composition

Milk production was initially measured at the end of the adaptation period ($\bar{X}$= day 11 of lactation), prior to reducing the temperature in the two chambers housing the CE ewes to 0 °C. Additional measurements were performed once weekly from wk 3 - 8 of lactation.

Milk yield was determined using an oxytocin-handmilking procedure. Between 0800 and 0900 h on the day of collection each lamb was confined to a wire cage within its dam's pen. This procedure had been previously tested with the animals and was determined to be the optimum method of separation to minimize the degree of stress experienced by both the ewe and lamb. Milk was collected by injecting 5 USP oxytocin IM and rapidly handmilking both halves of the udder. After milk flow had ceased, an additional 2.5 USP oxytocin was given IM and any residual milk stripped from the udder. The milk obtained from this first collection was discarded. The oxytocin-handmilking procedure was repeated 2.5 h later and the collected milk weighed to the nearest 0.1 g. Daily milk yield was estimated by multiplying the 2.5 h level of milk production by a factor of 9.6. Subsamples of the collected milk were immediately strained through cheesecloth, placed in sample vials containing potassium dichromate as a preservative and stored at 4 °C. Subsequently, percent fat, protein and lactose of each milk sample was determined in duplicate using infrared analysis (AOAC 1980).

Milk Energy Value

On the basis of milk yield and composition, the gross energy value of the milk was calculated according to the formula used by Kleiber (1975).

Experiment II

Animals

Ten yearling Suffolk-cross ewes nursing single lambs were selected shortly after parturition (X= 12 days) and assigned to one of two treatment groups, according to maternal bodyweight and sex of lamb. Ewes allotted to the control treatment group (CON) were housed within 1 of 2 temperature-controlled chambers maintained at 21±1 °C for 56 days (wk 2 - 10 of lactation) while the cold-exposed ewes (CE) were kept for the same period of time within 1 of 2 chambers maintained at an ambient temperature of 0±1 °C. Both treatment groups were subjected to continuous lighting. Each ewe-lamb pair was confined to a 0.9x1.8 m pen bedded with wood shavings. For periods of sample collection, a neck chain was used to tether the ewe;

during the remainder of the experimental period, movement within the pen was not restricted. A ration of 75 % rolled barley and 25 % alfalfa pellets was fed to each ewe in two equal allotments (0800 & 1600 h) at a level of 112 $g.kg^{-0.75}.day^{-1}$. Free access to water and cobalt-iodized salt was also provided. Bodyweight changes were monitored by weekly weighings.

At the beginning of the trial the ewes were treated for internal and external parasites and received intramuscular injections (mg) of retinol 75, cholecalciferol 0.94 and α-tocopherol 16. To ensure a relatively constant fleece depth of approximately 1 cm, the ewes were shorn at the beginning of the experiment and thereafter at 2 wk intervals.

Measurements

A summary of the manipulations made during this experiment is presented in Figure II.2.

Digestibility

During wk 7 of the experiment a digestibility marker, dysprosium (Dy), was added to the barley portion of the ration at the level of 20 ppm (elemental Dy). The marker, in the form of $DyCl_3.6H_2O$, was initially ground and mixed with an equivalent weight of finely ground barley, thoroughly mixed with 500 g of finely ground barley and then mixed with successive 5 kg increments of rolled barley until the final concentration was achieved. After an equilibration period of 3 days,

Figure II.2 Sequence of Animal Manipulations and Measurements, Experiment II.

Length on Trial		Stage of Lactation		Measurement						
Week	Days	Week	Days	Weight	Milk Production	Blood Sampling	Digestibility	Mammary Blood Flow	Organ Weights	
1	1	2	12	x						Adjustment Period
	2		13							
	3		14							
	4	3	15							
	5		16							
	6		17							
	7		18							
2	8		19	x						Differential Treatment Period
	9		20							
	10		21							
	11	4	22							
	12		23							
	13		24			x				
	14		25							
3	15		26		x					
	16		27							
	17		28							
	18	5	29	x						
	19		30			x				
	20		31							
	21		32		x					
4	22		33							
	23		34							
	24		35							
	25	6	36							
	26		37	x						
	27		38			x				
	28		39		x					
5	29		40							
	30		41							
	31		42							
	32	7	43							
	33		44	x						
	34		45			x				
	35		46		x					
6	36		47							
	37		48							
	38		49							
	39	8	50	x						
	40		51			x				
	41		52							
	42		53		x					
7	43		54							
	44		55							
	45		56	x						
	46	9	57			x				
	47		58				x			Dy Adaptation
	48		59				x			
	49		60				x			
8	50		61				x			Fecal Collection
	51		62							
	52		63					x		Surgery
	53	10	64					x		
	54		65					x		
	55		66					x		
	56		67	x				x	x	Microsphere Injection

during which the Dy-barley mixture was fed, 3 fecal grab samples were collected from each ewe over a 24 h period (0800, 1600, 2400 h). Samples of feed and feces were dried in a forced air oven at 65 °C to determine dry matter content. Other subsamples were freeze-dried and finely ground for neutron activation analysis. Duplicate samples consisting of approximately 1 g ground feces or 4 g ground barley were accurately weighed into 1.5 cm^3 polyethylene vials, activated by neutron bombardment in the University of Alberta SLOWPOKE facility and analyzed by γ-ray spectroscopy for ^{165}mDy content. Dry matter (DM) digestibility of the entire ration was estimated according to the following formula:

$$\text{DM dig \%} = 100-\left[100 \text{x} \frac{(\text{\%Dy in feed})}{(\text{\%Dy in feces})} \text{x} \frac{(\text{\%DM in feces})}{(\text{\%DM in feed})}\right]$$

Blood Samples

Blood samples were collected from each ewe once weekly from wk 2 - 7 of the experiment (wk 4 - 9 of lactation) for analysis of plasma concentrations of noradrenaline (NA), adrenaline (A), insulin, triiodothyronine (T_3), thyroxine (T_4) and glucose. Prior to (12-16 h) sample collection a polyethylene catheter was established in each ewe. The catheter was inserted through a 14G thinwall needle into a jugular vein and advanced approximately 10 cm before being fixed in position with an external skin suture. Between 0500 and 0600 h on the sampling day polyethylene extensions were attached to the jugular catheters and passed through portholes in the chamber walls. The animals were left undisturbed for the remainder of the sampling day except for feeding and occasional catheter adjustment. These procedures were used to minimize the degree of experimental stress experienced by the animals.

Blood samples were collected from outside of the chamber 1 h prior to and 1 h following each feeding (0700, 0900, 1500, 1700 h). At each sampling period a 20 ml aliquot of blood was collected into a plastic syringe containing 0.2 ml sodium heparin (1000 USP units/ml) for later analysis of insulin, T_3, T_4 and glucose. Additionally, a 5 ml blood sample was collected into a plastic syringe containing 100 ul glutathione/EGTA, as described by Pueler and Johnson (1977), for subsequent analysis of NA and A. The collected blood was placed into pre-chilled plastic test tubes and immediately centrifuged at 13000 g for 8 min at 4 °C. The separated plasma was subdivided into small plastic sample vials and stored at -40 °C prior to analysis.

Plasma samples collected during wk 3, 5 and 7 of the experiment were assayed for insulin using a ^{125}I insulin radioimmunoassay kit (Ammersham, Arlington Heights, Illinois) and for catecholamines (NA, A) using the radioenzymatic assay technique of Pueler and Johnson (1977), with some minor modifications as described by

Graham et al. (1981). Insulin concentrations determined for the sampling periods prior to and following the afternoon feeding (1500, 1700 h) were used to calculate an average plasma insulin concentration for each animal. Plasma NA and A concentrations for each animal were based on the analysis of plasma collected prior to the afternoon feeding (1500 h).

Plasma samples collected from wk 2 - 7 of the experiment were analyzed for levels of circulating T_3 and total (free+bound) T_4 using a ^{125}I T_3 radioimmunoassay kit and thyroxine coat-a-count kit, respectively (Inter-Medico, Willowdale, Ontario), and for glucose, using an automated neocuproine method as described by Bittner and Manning (1966). Individual mean plasma concentrations of T_3, T_4 and glucose were estimated for each collection day by averaging the results obtained from all 4 sampling periods (0700, 0900, 1500, 1700 h).

Milk Production and Composition

Milk production was estimated once weekly during wk 4 - 8 of lactation using a weigh-suckle-weigh technique. At 0700 h on the day of measurement the lamb was separated from the ewe by placing it in a wire cage within its dam's pen. At 0800 h the lamb was permitted to suckle and any milk remaining in the udder was stripped by hand. The lamb was then replaced in the cage and allowed to suckle at 3 h intervals over a total period of 24 h (Figure II.3). The quantity of milk produced at each suckling period was determined by weighing the lamb to the nearest gram immediately prior to and following nursing, the difference in weight being taken as the weight of milk consumed. Any feces or urine voided by the lamb during the suckling period were also collected and weighed. Daily milk production was estimated by totalling the results from the 8 separate suckling periods. Additionally, 24 h following the determination of milk yield, a milk sample was collected for analysis. By separating the ewe and lamb, milk was allowed to accumulate in the mammary gland over a 3 h period and was collected by injecting 5 USP oxytocin IV (jugular vein) and rapidly handmilking both halves of the udder. The milk samples were strained through cheesecloth to remove any debris. Subsamples were placed in vials containing potassium dichromate as a preservative and stored at 4 °C for 24 - 48 h prior to analysis in duplicate for fat, protein and lactose using infrared analysis (AOAC 1980). Separate duplicate 10 ml subsamples were freeze-dried to determine the total solids content of the milk or frozen and kept at -40 °C for later analysis of milk fatty acid composition.

Milk Fatty Acid Composition

Samples of milk fat for determination of fatty acid composition were extracted according to the method of Roese-Gottlieb (AOAC 1980). Following the mixture of

Figure II.3 Procedure for the Determination of Daily Milk Production Using the Weigh-Suckle-Weigh Technique.

Time	Procedure
0700 h	Lamb placed in wire cage
0800 h	Lamb allowed to suckle and udder stripped bare by hand Lamb replaced in cage
1100 h	Lamb weighed to nearest gram, allowed to suckle and reweighed before being replaced in cage. 3 h milk production: [post-suckling wt. + (urine & feces voided during suckling)] — pre-suckling wt.
1400 h	
1700 h	
2000 h	
2300 h	
0200 h	
0500 h	
0800 h	

Total milk production (24 h) = $\sum_{1}^{8}$ 3 h milk production.

10 ml of milk with 1.25 ml concentrated NH_4OH and 50 ml of a 40:40:20 (v:v:v) solution of diethyl ether, petroleum ether and ethanol in a separatory funnel, the ether layer was transferred to a 150x20 mm screw cap culture tube. Methyl esters of the fatty acids were prepared as described by Morrison and Smith (1964). After removing the solvent under nitrogen in a warm water bath, 15 ml of a 35:20:45 BF_3:pentane:methanol mixture was added to the tube and heated in a boiling water bath for 30 min. Equal proportions (v:v) of hexane and water were then added to the tube, thoroughly mixed and the hexane layer removed. The hexane solution of methyl esters was injected directly onto a Varian Model 3700 gas chromatograph fitted with a flame ionization detector and analyses carried out using quadrex gas capillary columns (60 m x 0.25 mm ID) coated with Silar 10C. Column temperature was held at 100 °C for 6 min, then programmed to rise at a rate of 12 °C/min to a final temperature of 198 °C, which was maintained for 20 min. Injector and detector temperatures were kept at 250 °C. The peak areas of the separated fatty acids were measured using a Hewlett-Packard 3353 Data System and identification of the peaks was made on the basis of retention time and comparison with known methyl esters. The twelve largest fatty acid peaks were chosen for measurement and these represented greater than 90 % of the total fatty acids in the milk.

Milk Energy

As in Experiment I, the gross energy value of the milk was estimated according to the formula used by Kleiber (1975).

Organ Weights

After injecting radionuclide labelled microspheres during wk 8 of the experiment to determine mammary blood flow (Chapter III), the ewes were stunned and bled to death. The digestive tract was removed, separated into its various parts and washed clean before being weighed. Additionally, the weights of the lungs, kidneys, mammary gland, thyroid and adrenal glands were determined for each animal.

Statistical Analysis

The results of Experiment I and II were analyzed separately. Treatment differences for each sampling period were compared using the Students t-test (Steel and Torrie 1980). Within a treatment group, data collected from several sampling periods were analyzed using Least Squares Analysis of Variance and where significant differences existed, means were compared using the Student-Newman-Keuls test of mean differences (Steel and Torrie 1980).

D. Results

Experiment I

Bodyweight

Mean bodyweight of both the CON and CE groups remained fairly constant throughout the experiment (Figure II.4).

Heat Production

On the basis of heat production, the ewes housed in the cold were kept at a temperature below their thermoneutral zone; heat production measured after 24 h, 4 wk and 7 wk of cold exposure respectively, was 0.42 kcal.kg^{-1}.h^{-1} ($P<0.05$), 0.92 kcal.kg^{-1}.h^{-1} ($P<0.01$) and 1.27 kcal.kg^{-1}.h^{-1} ($P<0.001$) higher in the CE ewes compared to the control animals (Table II.2). While the heat production of the CON ewes did not vary over the course of lactation ($P>0.10$), heat production in the CE ewes rose ($P<0.05$) with increasing duration of the experiment.

Milk Production

Milk production (g/day) results are shown in Table II.3 and Figure II.5. Milk yield measured during the pre-treatment period was similar for both treatment groups. The development of mastitis in 2 CE ewes and 1 CON ewe near the end of the trial reduced the number of animals available for analysis of milk production. Milk yield did not differ between groups ($P>0.10$) except for wk 5 of lactation when milk production tended to be

Figure II.4 Change in Bodyweight of the Control (●——●) and Cold-Exposed (o– – –o) Ewes, Experiment I.

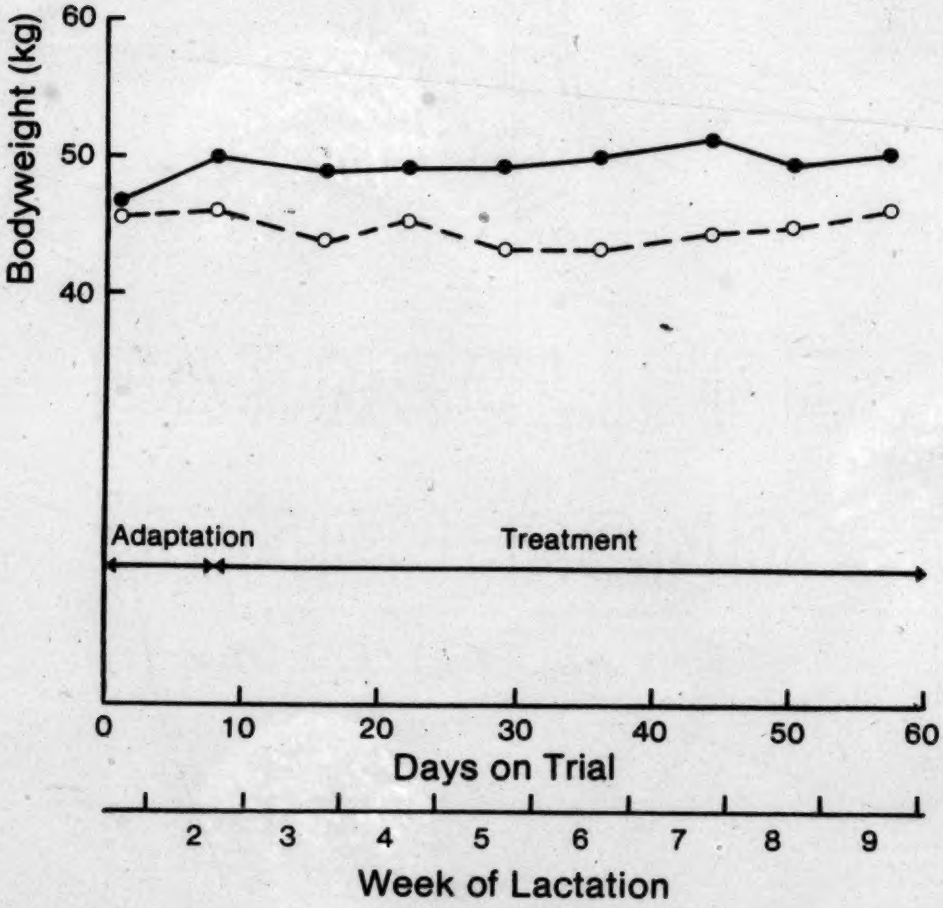


Table II.2 Heat Production of the Control and Cold-Exposed Ewes, Experiment I

Stage of Lactation (wk)	Duration of Cold Exposure (days)	Heat Production[1] (kcal.kg^{-1}.h^{-1}) CON[2] (4)	CE (4)
2	1	2.12±0.10*	2.54±0.12*a*
5	21	2.16±0.07**	3.08±0.16*b*
8	41	2.30±0.10‡	3.57±0.08*c*
	$\bar{X}$=	2.19±0.05	3.06±0.14

[1] values represent mean±SEM.

[2] number of animals/treatment in parentheses.

a,b,c means within a column followed by a different letter are significantly different ($P<0.05$).

* treatment means are significantly different ($P<0.05$).

**treatment means are significantly different ($P<0.01$).

‡ treatment means are significantly different ($P<0.001$).

Table II.3 Daily Milk Production of the Control and Cold-Exposed Ewes, Experiment I

	Milk Production (g/day)[1]	
Stage of Lactation	CON (4)[2]	CE (4)
2(pretreatment)	1661.8±178.8	1576.3±211.0
3	1585.9± 69.1	1894.6±214.8
4	1671.0±103.2	1587.6± 73.8
5	1797.4± 92.1	1482.2±196.8+
6	1441.0±148.4	1555.2±175.0[3]
7	1253.1± 80.2[3]	1307.9± 68.7[3]
8	1398.2± 65.8[3]	1112.2± 35.0[4]*
Overall Mean	1560.9± 51.7	1540.6± 67.8

[1] values represent mean±SEM.

[2] number of animals/treatment in parentheses.

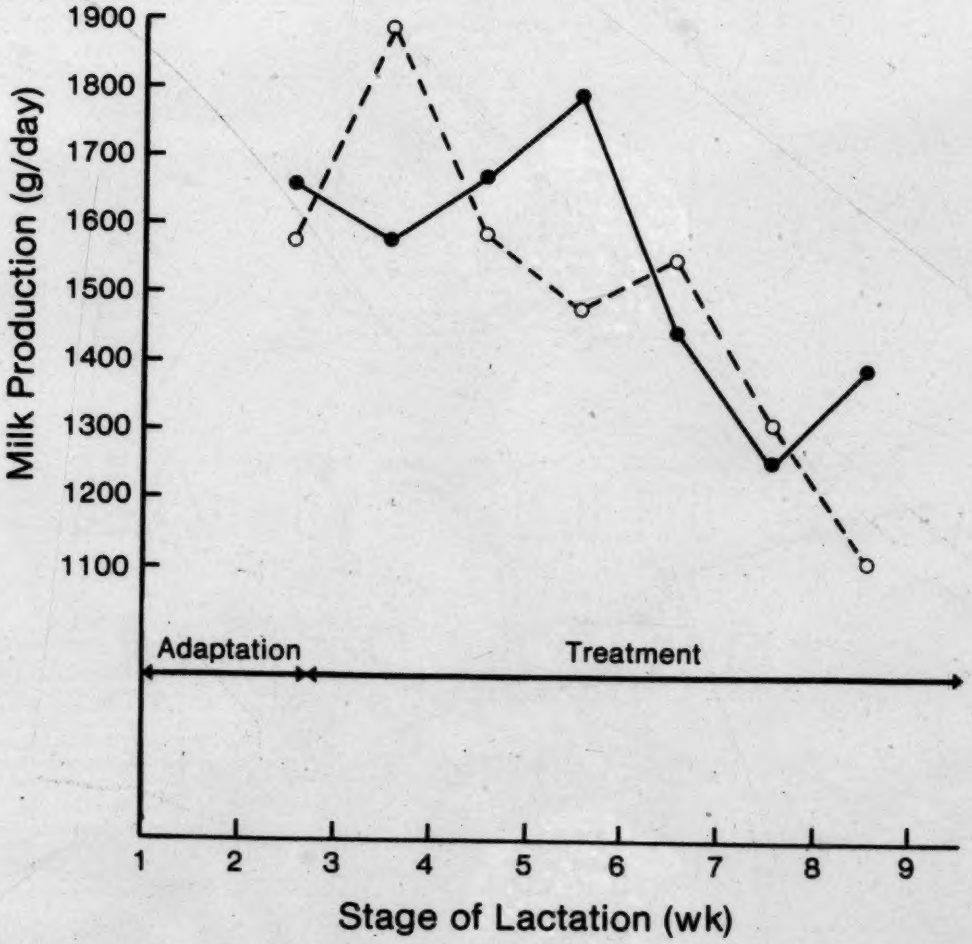
[3] value represents mean±SEM of 3 animals.

[4] value represents mean±SEM of 2 animals.

+ treatment means are significantly different (P<0.06).

* treatment means are significantly different (P<0.05).

Figure II.5 Daily Milk Production of the Control (●——●) and Cold-Exposed (○– – –○) Ewes, Experiment I.



depressed in the CE ewes (P<0.06) and during wk 8 of lactation when milk yield measured in 2 CE ewes was lower (P<0.05) than that measured in 3 CON animals.

Milk Composition

Analysis of the composition of milk revealed a tendency towards increased milk fat concentration in the cold-exposed ewes (Table II.4). Milk fat concentration tended to increase in both groups of animals over the course of lactation, although the trend was more pronounced in the cold-exposed ewes.

Despite a slightly lower pre-treatment concentration of milk protein in the CE ewes (P<0.10), protein concentration measured during the treatment period was similar for both groups of animals.

The lactose concentration of milk peaked at mid-lactation and thereafter declined in both treatment groups however treatment differences in percent milk lactose were not apparent (P>0.05).

Milk Energy Value

Since total daily milk fat secretion was increased while protein and lactose secretions were maintained during cold exposure, the energy output in milk was similar for both treatment groups but occasionally was slightly higher (wk 3 of lactation P<0.05; wk 7 of lactation P<0.10) in the CE animals (Table II.5).

Table II.4 Comparison of the Mean Concentrations of Fat, Protein and Lactose in the Milk of the Control and Cold-Exposed Ewes, Experiment I[1]

Stage of Lactation (wk)	Fat (%)		Protein (%)		Lactose (%)	
	CON (4)[2]	CE (4)	CON (4)	CE (4)	CON (4)	CE (4)
2	8.56±1.11	8.60±0.99	5.07±1.11 *ab*	4.50±0.24 *a*†	5.16±0.05 *a*	5.20±0.16 *a*
3	6.48±0.62	9.10±1.71	4.51±0.10 *a*	4.20±0.14 *a*	5.62±0.08 *ab*	5.71±0.13 *b*
4	6.65±1.77	9.57±0.41	4.57±0.37 *a*	4.77±0.26 *ab*	5.94±0.05 *b*	5.81±0.10 *b*
5	8.50±1.27	9.04±1.24	5.15±0.15 *ab*	5.44±0.11 *ab*	5.94±0.10 *b*	5.83±0.13 *b*
6	6.71±0.72	7.77±0.31[3]	5.69±0.18 *b*	5.17±0.75[3]*ab*	5.47±0.14 *ab*	5.89±0.09[3]*b*†
7	7.93±1.29[2]	9.56±0.61[3]	5.40±0.21[3]*ab*	5.42±0.10[3]*ab*	5.56±0.08[3]*ab*	5.77±0.12[3]*b*
8	7.08±0.63[3]	9.64±0.62[4]‡	5.41±0.17[3]*ab*	6.09±0.36[4]*b*	5.51±0.31[3]*ab*	5.36±0.02[4]*ab*
Overall Mean	7.41±0.42	9.02±0.38	5.09±0.11	4.98±0.15	5.60±0.07	5.66±0.07

[1] values represent mean±SEM.
[2] number of animals/treatment in parentheses.
[3] value represents mean of 3±SEM animals.
[4] value represents mean of 2±SEM animals.
a,b means within a row followed by a different letter are significantly different (P<0.05).
† treatment means are significantly different (P<0.10).
‡ treatment means are significantly different (P<0.001).

Table II.5 Gross Energy Value of the Milk Obtained From the Control and Cold-Exposed Ewes[1], Experiment I

	Milk Energy (Mcal/day)[2]	
Stage of Lactation (wk)	CON (4)[3]	CE (4)
2(pretreatment)	2.19±0.381	1.94±0.199
3	1.72±0.145	2.42±0.219*
4	1.90±0.375	2.21±0.133
5	2.36±0.202	2.02±0.054
6	1.68±0.188	1.94±0.201[4]
7	1.57±0.102[5]	1.86±0.092[4]†
8	1.65±0.064[4]	1.62±0.010[5]
Overall Mean	1.89±0.102	2.04±0.071

[1] Milk E(mcal/day)=fat(g/day)x9.20x10^{-3}mcal/g+protein(g/day)x5.86x10^{-3}mcal/g+lactose(g/day)x3.95x10^{-3}mcal/g.
[2] values represent mean±SEM.
[3] number of animals/treatment in parentheses.
[4] value represents mean±SEM of 3 animals.
[5] value represents mean±SEM of 2 animals.
† treatment means are significantly different ($P<0.10$).
* treatment means are significantly different ($P<0.05$).

Experiment II

Bodyweight

As in Experiment I, the mean bodyweight of both treatment groups remained relatively constant throughout the experiment (Figure II.6).

Digestibility

Based on measurements performed during the ninth week of lactation, after 7 wk of differential temperature treatment, ration DM digestibility averaged 88.2±0.62 % in the CON animals but only 84.4±1.47 % ($P<0.05$) in the CE ewes.

Blood Samples

Hormone profiles of the animals are presented in Table II.6. Plasma concentration of NA was consistently higher in the CE ewes but showed a tendency to decrease over the period of cold exposure ($P<0.05$). NA concentration measured during the third and seventh weeks of the experiment was significantly higher in the CE group ($P<0.05$), although a treatment difference was not observed during the fifth week of the trial ($P>0.10$).

Plasma concentration of A also tended to be elevated during the third and seventh weeks of the experiment in the CE ewes, although the mean level measured at wk 5 of the experiment was inexplicably lower than that measured in the CON animals. None of the treatment differences observed over the experimental

Figure II.6 Change in Bodyweight of the Control (●——●) and Cold-Exposed (○- - -○) Ewes, Experiment II.

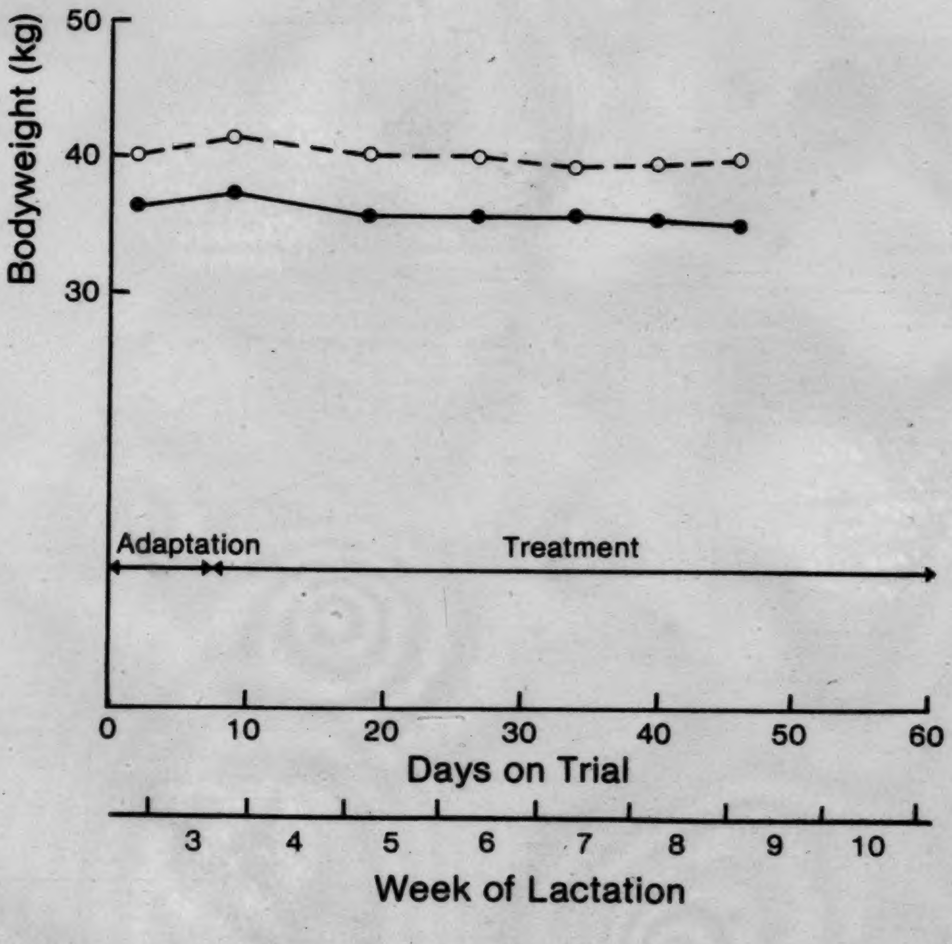


Table II.6 Comparison of the Mean Concentrations of Various Hormones and Glucose in the Plasma of the Control and Cold-Exposed Ewes, Experiment II[1]

Stage of Lactation (wk)	4	5	6	7	8	9
Stage of Trial (wk)	2	3	4	5	6	7
Noradrenaline (pg/ml):						
CON		237.0± 71.98*		362.0± 97.62		75.7±22.67*
CE		1083.3±255.20*a*		454.0±150.44*b*		211.7±42.40*b*
Adrenaline (pg/ml):						
CON		32.8±13.54		43.5±38.50		26.5±11.69
CE		136.3±63.13		11.0± 3.00		43.3±15.96
Insulin (uU/ml):						
CON		72.8± 8.29		36.4±2.44		50.4± 7.87
CE		72.4±15.01		36.0±7.73		56.7±11.66
T 3 (ng/100ml):						
CON	104.0±12.20	113.3±22.48	129.0±22.15*	145.1±26.66*	135.7±15.10**	148.8±15.05**
CE	107.7±11.00*a*	122.2±25.90*a*	249.6±33.04*b*	232.6±22.45*b*	225.1±16.59*b*	239.1±13.76*b*
T 4 (ug/100ml):						
CON	3.95±0.43*	3.86±0.88	5.09±1.27	8.14±1.61	7.51±1.20	7.89±1.39†
CE	2.14±0.30*a*	3.06±0.52*a*	7.24±0.89*b*	9.76±0.95*c*	9.50±0.53*c*	10.80±0.72*c*
Glucose (mg/dl):						
CON	69.9±3.98†	66.3±4.45	66.0±2.57*	61.9±2.09	63.8±3.92	59.3±3.70
CE	80.3±3.52	74.1±5.81	79.0±3.84	62.4±5.40	67.4±5.61	68.9±3.90

[1] values represent mean±SEM.

a,b,c means within a row followed by a different letter are significantly different ($P<0.05$).

† treatment means are significantly different ($P<0.10$).

* treatment means are significantly different ($P<0.05$).

**treatment means are significantly different ($P<0.01$).

period were statistically significant ($P>0.10$).

Insulin concentration in plasma was similar in both treatment groups but tended to be lowest during mid-lactation.

T_3 plasma levels rose during the period of cold exposure and were significantly higher than in the control group after 3 wk of treatment ($P<0.05$). T_3 concentration measured in the CON ewes was relatively stable over the course of lactation. In contrast, plasma T_4 concentration tended to be lower in the CE group during the first 2 wk of cold exposure but rose and remained elevated during the remainder of the experimental period ($P<0.05$). Plasma T_4 concentration measured after 6 wk of cold exposure tended to be higher in the CE ewes ($P<0.10$), although initial plasma T_4 levels were slightly lower ($P<0.10$) in the cold-exposed animals.

Concentration of glucose in the plasma was consistently greater in the CE ewes but was significantly higher ($P<0.05$) only during wk 4 of lactation.

Milk Production

Milk production (g/day) results are shown in Table II.7 and Figure II.7. The milk production of both treatment groups in Experiment II was markedly lower than during Experiment I. Development of hypothermia in 1 CE ewe during wk 4 of lactation resulted in the

Table II.7 Daily Milk Production of the Control and Cold-Exposed Ewes, Experiment II

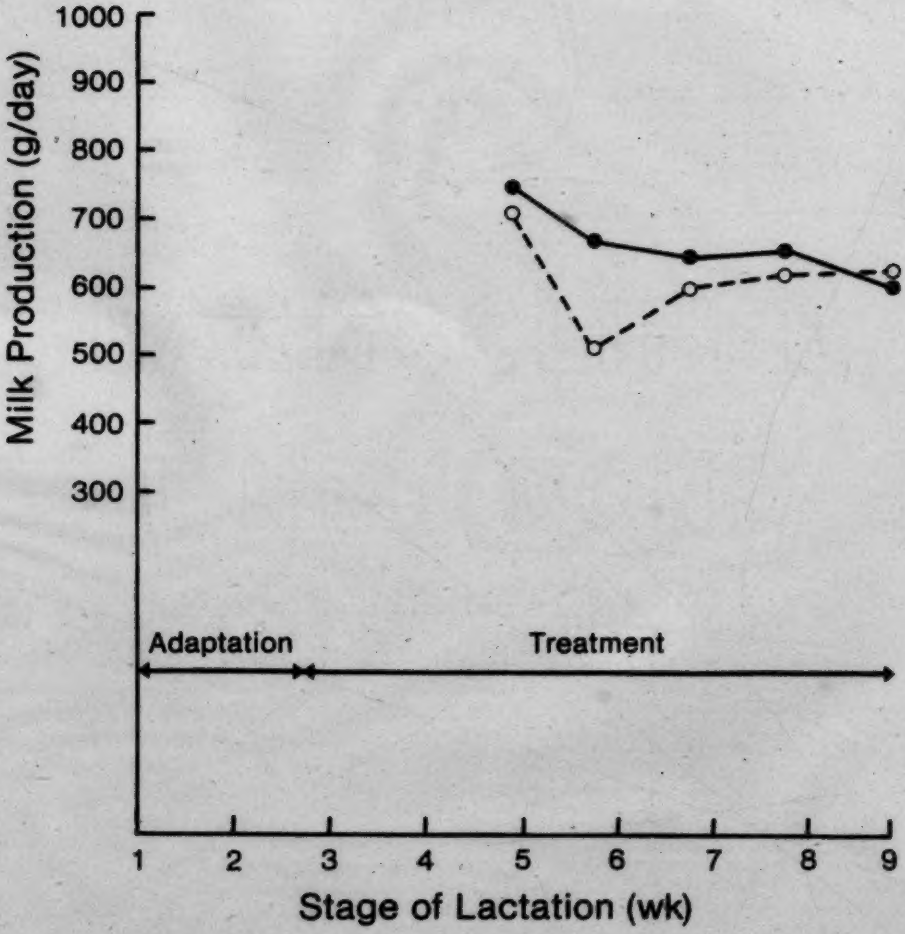
	Milk Production (g/day)[1]	
Stage of Lactation (wk)	CON (5)[2]	CE (5)
4	752.6± 60.9	718.8±100.7[3]
5	677.0± 55.7	516.0±109.8
6	656.8± 94.7	601.6±109.7
7	662.4±102.0	630.2±120.5
8	604.8±104.1	635.6±101.6
Overall Mean	670.7± 36.5	616.3± 46.6

[1] values represent mean±SEM.

[2] number of animals/treatment in parentheses.

[3] value represents mean±SEM of 4 animals.

Figure II.7 Daily Milk Production of the Control (●——●) and Cold-Exposed (○– – –○) Ewes, Experiment II.



removal of the ewe from the experiment for a 24 h period; consequently, mean milk production during this one period reflects the results obtained from 4 CE and 5 CON ewes. Although milk production tended to be slightly lower in the CE ewes, the effect of temperature was not significant.

Milk Composition

Percentage milk fat tended to be elevated in the CE group (wk 5, $P<0.10$; wk 7, $P<0.01$) (Table II.8) and rose slightly in both groups of animals over the course of lactation.

Milk protein concentration also tended to be elevated in the cold-exposed animals and was significantly greater ($P<0.05$) during wk 7 of lactation, after 5 wk of cold exposure.

Except for a tendency of increased milk lactose concentration during the first week of measurement (after 2 wk cold exposure) treatment differences in percent milk lactose were not apparent ($P>0.10$).

Milk Fatty Acid Composition

From the analysis of the fatty acid composition of milk fat (Table II.9) it is evident that cold stress resulted in changes in mammary fatty acid metabolism. Statistical analysis failed to reveal any change in fatty acid composition over time for either treatment group ($P>0.05$), therefore, the results from 5 wk of sample collection have been pooled. In the CON and CE

Table II.8 Comparison of the Mean Concentrations of Fat, Protein and Lactose in the Milk of the Control and Cold-Exposed Ewes, Experiment II[1]

Stage of Lactation (wk)	Fat (%)		Protein (%)		Lactose (%)	
	CON (5)[2]	CE (5)	CON (5)	CE (5)	CON (5)	CE (5)
4	7.28±0.56*a*	6.53±0.31[3]*a*	4.62±0.09	4.73±0.19[3]	5.30±0.08	5.85±0.03[3]†
5	6.34±0.27*a*	9.18±1.32*ab*†	4.76±0.15	5.12±0.12	5.38±0.06	5.66±0.18
6	6.78±0.72*a*	7.44±0.37*ab*	4.72±0.16	5.50±0.48	5.64±0.07	5.80±0.21
7	7.68±0.32*a*	10.44±1.49*b***	4.82±0.15	5.60±0.31*	5.52±0.10	5.52±0.12
8	10.40±1.62*b*	13.46±1.04*c*	5.10±0.20	5.82±0.31	5.46±0.10	5.30±0.13
Overall Mean	7.70±0.45	9.53±0.62	4.80±0.07	5.38±0.15	5.46±0.04	5.62±0.08

[1] values represent mean±SEM.
[2] number of animals/treatment in parentheses.
[3] value represents mean±SEM of 4 animals.
a,b,c means within a row followed by a different letter are significantly different ($P<0.05$).
† treatment means are significantly different ($P<0.10$).
* treatment means are significantly different ($P<0.05$).
**treatment means are significantly different ($P<0.01$).

Table II.9 Fatty Acid Composition of the Milk Obtained From the Control and Cold-Exposed Ewes, Experiment II'

Fatty Acid:	Percent of Total Milk Fatty Acids CON	 CE	P Level
C4:0	1.73±0.101	1.34±0.085	P<0.01
C6:0	2.53±0.145	1.99±0.094	P<0.01
C8:0	3.14±0.240	2.40±0.145	P<0.01
C10:0	9.89±0.823	7.91±0.554	P<0.05
C12:0	5.50±0.473	4.26±0.312	P<0.05
C14:0	10.08±0.398	8.42±0.479	P<0.05
C16:0	25.20±0.765	26.18±0.619	
C16:1	2.02±0.119	2.02±0.073	
C18:0	9.64±0.723	11.24±0.452	P<0.06
C18:1(trans)	3.27±0.426	3.02±0.345	
C18:1(cis)	22.82±1.521	26.48±1.409	P<0.10
C18:2	4.19±0.244	4.33±0.317	
short chain fatty acids (<16C)	32.87±1.831	26.33±1.509	P<0.01
long chain fatty acids (≥16C)	67.13±1.824	73.25±1.465	P<0.02

' values represent mean±SEM of data collected from 5 animals/treatment over 5 weeks of lactation.

ewes respectively, short chain fatty acids (less than 16 C in length) formed 32.9 % and 26.3 % of the total milk fatty acids ($P<0.01$) whereas the proportion of long chain fatty acids (greater than or equal to 16 C in length) was respectively, 67.1 % and 73.3 % ($P<0.02$). Alterations in fatty acid compostion reflected lower percentages of C4:0, C6:0, C8:0 ($P<0.01$), C10:0, C12:0, and C14:0 ($P<0.05$) fatty acids but higher proportions of C18:0 ($P<0.06$) and C18:1(cis) ($P<0.10$) fatty acids in the milk collected from the CE ewes.

Milk Energy Value

Since the cold temperature treatment employed in this experiment had little effect on milk production or composition, the energy content of the milk was calculated to be similar for both treatment groups (Table II.10).

Organ Weights

Measurements of organ weights revealed few treatment effects (Table II.11) although the weight of the mammary gland, expressed in terms of g/kg BW, was reduced ($P<0.05$) and the weight of the adrenal glands relative to bodyweight was increased by 25.8 % ($P<0.05$) as a result of cold exposure.

Efficiency of Milk Production

From a compilation of results presented in this and in the following chapter, the efficiency of milk production was estimated (Table II.12). While total

Table II.10 Gross Energy Value of the Milk Obtained From the Control and Cold-Exposed Ewes[1], Experiment II

Stage of Lactation (wk)	Milk Energy (Mcal/day)[2]	
	CON (5)[3]	CE (5)
4	0.86±0.053	0.79±0.094[4]
5	0.72±0.045	0.73±0.217
6	0.73±0.082	0.74±0.135
7	0.79±0.106	0.94±0.174
8	0.93±0.244	1.11±0.143
Overall Mean	0.80±0.055	0.87±0.073

[1] Milk E(mcal/day)=fat(g/day)x9.20x10 'mcal/g+protein(g/day)x5.86x10 'mcal/g+lactose(g/day)x3.95x10 'mcal/g.
[2] values represent mean±SEM.
[3] number of animals/treatment in parentheses.
[4] value represents mean±SEM of 4 animals.

Table II.11 Organ Weights of the Control and Cold-Exposed Lactating Ewes, Experiment II[1]

Organ Weight (g/kg BW)	CON (5)[2]	CE (5)
Digestive Tract:		
Reticulum	4.58±0.78	4.03±0.14
Rumen	23.36±1.61	24.79±2.67
Omasum	3.51±0.32	2.82±0.25
Abomasum	6.89±0.59	6.77±0.42
Duodenum	0.77±0.20	0.94±0.05
Jejunum & Ileum	16.92±1.74	16.33±2.06
Cecum	1.93±0.16	1.96±0.24
Colon	15.23±1.80	13.76±1.37
Lungs	11.72±0.79	11.43±0.88
Kidneys	3.53±0.20	3.15±0.11
Mammary Gland	14.72±0.74	10.61±1.33*
Endocrine Glands:		
Thyroid	0.112±0.021	0.129±0.021
Adrenals	0.120±0.004	0.151±0.011*

[1] values represent mean±SEM.

[2] number of animals/treatment in parentheses.

* treatment means are significantly different (P<0.05).

Table II.12 The Efficiency of Milk Production of the Control and Cold-Exposed Ewes, Experiment II[1]

Measurement:	CON	CE
Total Mammary Weight[2] (g)	502.6± 32.57	426.6± 87.41
Total Secretory Tissue Weight[3] (g)	332.5± 29.64	288.0± 51.60
Milk Secretion[4] (g/day)	604.8±104.14	635.6±101.56
Efficiency of Milk Production:		
g milk/g mammary tissue	1.22 ± 0.24	1.56 ± 0.11
g milk/g secretory tissue	1.84 ± 0.76	2.45 ± 0.19
Total Mammary Blood Flow[5] (ml/min)	301.4± 68.69	176.0± 52.00
Milk Secretion[6] (ml/min)	0.410± 0.070	0.431± 0.068
Blood Flow:Milk Yield Ratio	664.0±179.23	359.7±109.98

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[1] values represent mean±SEM.

[2] mammary weight determined after 56 days of treatment (10 wk of lactation) in 5 CON and 5 CE ewes.

[3] secretory tissue weight based on dissection data obtained from the right half of the mammary gland of 5 CON and 4 CE ewes:
total secretory wgt.=(total udder wgt)x(right secretory wgt./right udder half wgt.).

[4] milk secretion data obtained during wk 8 of lactation (day 42 of the experiment) in 5 CON and 5 CE ewes.

[5] total mammary blood flow based on blood flow to the right half of the udder, measured during wk 10 of lactation (day 56 of the experiment).

[6] volume of milk secretion= wgt of milk x specific gravity

mammary blood flow and mammary and secretory tissue weights, measured during the tenth week of lactation, tended to be reduced as a result of chronic cold exposure, milk production, assessed during the eighth week of lactation, was slightly greater in the CE ewes. Efficiency of milk production expressed as g milk/g mammary tissue, g milk/g secretory tissue and blood flow:milk yield respectively, was calculated to be 1.22, 1.84 and 664.0 for the CON ewes and 1.56, 2.45 and 359.7 for the CE ewes.

E. Discussion

Heat Production

Throughout lactation, Degen and Young (1982) determined that thermoneutral heat production (Hp) in the ewe ranged from 1.98 to 2.19 kcal.kg^{-1}.h^{-1} , peak values occuring at wk 2 - 4 of lactation. The heat production data for the control ewes in the present experiment (Experiment I) closely agrees with Degen and Young's (1982) results. Although the trend was not statistically significant, the metabolic rate of the ewes housed at a neutral temperature tended to rise from an initial value of 2.12 kcal.kg^{-1}.h^{-1} to a level of 2.30 kcal.kg^{-1}.h^{-1} , after 7 weeks of lactation. The rise in metabolic rate over the experimental period however, was not mirrored by a similar increase in milk production.

Since lactation in the ewe induces a 50 - 62 % increase in metabolic rate (Degen and Young 1982), the lower critical temperature of the lactating ewe would be below that of a non-pregnant-non-lactating animal. Thompson (1980) reported that the exposure of non-lactating ewes to 0 °C elicited a 45 % increase in metabolic rate, confirming that 0 °C is below the lower critical temperature of a shorn ewe at maintenance. From the current experiment however, it appears that an ambient temperature of 0 °C was also below the lower critical temperature of the lactating ewes examined; metabolic rate of the ewes exposed to 0 °C for 24 h was elevated by 20 % over the control value, indicating that the ewes were required to increase their heat production above that normally associated with lactation in order to maintain a state of homeothermy. Part of the increase in Hp observed in the CE ewes is also due to their higher feed consumption and associated heat increment of feeding. According to Kleiber's (1975) estimates, the heat increment due to the extra feed consumed by the CE ewes in the present experiment would only account for an increase in Hp of 0.039 $\text{kcal.kg}^{-1}.\text{h}^{-1}$, or less than 2 % of the total Hp of the CON lactating animals.

As the length of cold exposure increased, Hp measured after 21 and 41 days of cold exposure, respectively, was 143 % and 155 % of the control values. The rise in metabolic rate of the chronically cold-stressed ewes presumably is evidence of the development of metabolic acclimation, similar to the process that occurs in sheep exposed to winter conditions (Webster et al. 1969). As well, body fat in the CE ewes may have been increasingly lost over the course of the experiment, resulting in less insulation and a greater susceptibility to cold. This possibility was not directly examined in the present experiment and was not reflected by any change in bodyweight, although it is suggested by the changes in fatty acid composition of the milk.

Net Energy Balance

From the measurements of heat production and milk energy output, the total energy expenditure of the animals in Experiment I was estimated and compared to their metabolizable energy intake, calculated from published estimates of the ME value of barley and alfalfa pellets for sheep (NRC 1975)(Table II.13). Although the data collected during Experiment II would suggest that the DM digestibility of the barley-alfalfa diet fed in Experiment I would be reduced, and urinary energy loss has been reported to increase (Graham et al. 1959), methane energy losses are reduced as a result of cold exposure (Kennedy and Milligan 1978). These factors complicate the prediction of the ME content of a diet fed to cold-stressed animals, consequently the ME value of the diet was assumed to be equivalent for both treatment groups. On the basis of these assumptions, the calculated net energy balance of the ewes in Experiment I indicated a greater negative energy balance for the CE ewes, suggesting that the cold-stressed ewes must have relied on the mobilization of body stores to meet their maintenance and lactational requirements to a greater extent than the CON animals.

Digestibility

Apparent DM digestibility of the 75 % barley-25 % alfalfa ration fed to the lactating ewes in Experiment II was reduced by 0.19 digestibility units/°C. This reduction in digestibility agrees well with the reported depression in digestibility of .1-.3 units/°C for a roughage based diet fed to cold-stressed mature ruminants (see NRC 1981) and for a 50 % concentrate diet fed to cold-stressed calves and steers (Christopherson 1976). In

Table II.13 Partitioning of the Energy Requirements of the Control and Cold-Exposed Ewes, Experiment I

	CON Week of Lactation 2	 5	 8	CE Week of Lactation 2	 5	 8
Energy Intake (mcal/day)	5.73	5.13	5.13	5.73	5.73	5.73
Heat Production (mcal/day)	3.06	3.07	3.29	3.42	3.96	4.70
Milk Energy Content (mcal/day)	2.19	2.36	1.65	1.94	2.02	1.62
Total Energy Requirement (mcal/day)	5.25	5.43	4.94	5.36	5.98	6.32
Net Energy Balance (mcal/day)	+0.48	-0.30	+0.19	+0.37	-0.25	-0.59

contrast, Young and Degen (1981) did not observe any effect of a cold environment on the digestibility of a 50 % concentrate-50 % pelleted hay diet fed to mature wethers nor did Kennedy et al. (1982) find any detrimental effect of low temperature on the digestibility of a 100 % concentrate ration fed to mature wethers. The observed influence of cold exposure on the digestibility of a barley-based ration in lactating ewes may represent a combined environment-diet-physiological status interaction, however additional work is required to investigate this hypothesis. As well, the use of dysprosium as a digestibility marker in sheep needs to be validated by further research.

Hormones

Catecholamines

When care has been taken to ensure that the animals are not stressed at the time of blood sampling, plasma levels of NA and A, respectively, in mature wethers housed at a thermoneutral temperature have been reported to be 0.31 and 0.08 ng/ml (Christopherson et al. 1978); 0.24 and 0.07 ng/ml (Thompson et al. 1978b) and 0.24 and 0.04 ng/ml (Graham et al. 1981). Our values of NA and A in the non-stressed lactating ewe correspond well with these previous results.

In the current experiment, three weeks of cold exposure resulted in a 4.5-fold increase in plasma NA concentration and a 4.2-fold increase in plasma A concentration compared to control levels. Because of the small number of animals and the large degree of individual variation, the increase observed in A concentration was not statistically significant. As the duration of cold exposure increased, both A and NA levels in the cold-stressed animals fell; by the end of the experiment, plasma A levels were similar in both treatment groups, although NA concentration remained significantly elevated in the cold-stressed animals.

Intravenously infused A and NA have a thermogenic effect in sheep (Graham and Christopherson 1981) confirming that catecholamines likely play an important calorigenic role in the response of this species to cold stress. The effect of exogenous A seems to occur at physiological levels however supra-physiological levels of NA are required to elicit a response. Since NA is largely released at nerve endings rather than into the bloodstream it is uncertain that the establishment of effective concentrations of NA at tissue receptor sites by IV infusion can mimic the endogenous release of NA.

Although greatly elevated levels of plasma A and NA have been reported in mature sheep during acute cold exposure (Thompson et al. 1978b) and after 4 weeks of exposure to 2-5 °C (Christopherson et al. 1978), it is possible that plasma catecholamine concentrations are not maintained at such a high level over a longer period of cold stress. In rats exposed to 5 °C for 4 weeks, Leduc (1961) observed an initial 5-fold increase in urinary A excretion followed by a decline to near pre-exposure levels. Leduc (1961) and Shum et al. (1969) have demonstrated an initial peak in urinary excretion of NA in cold-stressed rats during the first few weeks of treatment, after which a substantial decline occured. In contrast, Shum et al. (1969) did not find any reduction in urinary A excretion in the rat over 4 weeks of cold exposure nor did Graham et al. (1981) observe any decrease in plasma concentrations of A and NA in wethers continuously exposed to 8 - 9 °C for 44 days.

A decrease in circulating levels or urinary excretion of catecholamines has been postulated to be involved in the process of metabolic acclimation to a chronic cold stress. Graham and Phillips (1981) have shown that the hyperglycemic response to A in sheep is potentiated by prolonged cold exposure, as is the thermogenic effect of A (Graham and Christopherson 1981). Similar changes in sensitivity to NA as a result of cold exposure apparently do not occur in sheep (Graham and Christopherson 1981; Graham and Phillips 1981), although daily NA injection in the rat increases its calorigenic effect (Leblanc and Pouliot 1964). It is possible then that the reduction in the level of plasma catecholamines, especially of A, during a prolonged period of cold exposure is associated with a sensitization of the lactating ewe to the effects of these hormones.

Insulin

The effects of cold exposure on plasma levels of insulin in sheep have not been clearly defined. While Bassett and Alexander (1971) reported that plasma insulin concentration in lambs generally declined during acute cold exposure, Thompson et al. (1978a) did not find any effect of low temperature on portal vein levels of insulin in adult sheep measured from 4 min to 5 h after feeding, although levels measured just prior to a meal were elevated in the cold-exposed animals.

During lactation, the shift in metabolism to support milk production may alter the hormonal response to cold exposure. Faulkner et al. (1980) determined that lactating goats acutely exposed to a cold environment, tended to show elevated plasma levels of insulin during certain portions of the day but that during the remainder of time, plasma insulin concentration in the cold-stressed animals paralleled that in the control goats. In the present experiment the effect of chronic cold stress was not reflected by any change in plasma insulin concentration in the lactating ewe.

Thyroid Hormones

The importance of thyroid hormones in establishing an optimum level of metabolism in many tissues has led to considerable investigation as to the role of the thyroid gland during periods of increased metabolic rate.

In mature sheep housed at a neutral temperature, plasma levels of T_3 and T_4 respectively, of 94.7 ng/100 ml and 8.62 ug/100 ml (Westra and Christopherson 1976); and 38 ng/100 ml and 7.2 ug/100 ml (Christopherson et al. 1978) have previously been reported. In the present experiment, plasma concentrations of T_3 and T_4 respectively, averaged 129.0 ng/100 ml and 5.94 ug/100 ml in the CON ewes.

Gale (1973) stated that acclimation to the cold likely involved a synergistic action between thyroid hormones and catecholamines. From a review of the literature, Thompson (1977) concluded that, although many studies had examined the influence of low temperature on circulating levels of T_3 and T_4, the results were controversial. Increased plasma T_3 and T_4 levels in wethers after prolonged cold exposure (4 - 6 weeks) have been reported by Westra and Christopherson (1976) and Christopherson et al. (1978). In the present experiment, circulating levels of T_3 in the cold-stressed lactating ewes were increased after 3 weeks of cold exposure and remained significantly elevated for the duration of the experiment. In contrast, T_4 levels were lower in the CE ewes after 1 week of cold exposure in comparison to the CON ewes although during the remainder of the experiment, T_4 levels tended to be higher in the CE animals. Thompson (1977) suggested that plasma concentration of T_3 may increase more than that of T_4 during cold exposure, perhaps reflecting the preferential secretion of T_3, the more calorigenic of the two thyroid hormones, or the increased conversion of T_4 to T_3 in the peripheral tissues. Since, in the present experiment, plasma T_3 levels were more indicative of cold exposure than were T_4 levels, our results would tend to support this theory.

Glucose

Plasma glucose concentration in the CE ewes consistently was elevated over that measured in the CON ewes, however the difference between treatments was not always statistically significant. Similarly, plasma glucose levels have been shown to increase in cold-stressed lactating goats (Faulkner et al. 1980) and in mature sheep (McKay et al. 1974; Christopherson et al. 1978; Thompson et al. 1978b), although Graham and Phillips (1981) were unable to detect any change in plasma glucose in chronically cold stressed wethers.

Milk Production

The milk production data collected during Experiment I is in broad agreement with other published reports of milk yield in the ewe nursing a single lamb (McCance 1959; Coombe et al. 1960; Gardner and Hogue 1964; Davis et al. 1978; Torres-Hernandez and Hohenboken 1979,1980) and closely agrees with Degen and Young's (1982) estimate of milk production in ewes obtained from the same flock, based on water turnover in the suckling lambs. The level of milk production measured during Experiment II however is lower than previously published values. The discrepancy between the two current experiments may reflect factors such as genetic variation, age of the ewes, number of lactations and bodyweight or could be a result of the technique employed to estimate milk production.

Studies of milk yield in the ewe and in other animals not accustomed to daily hand or machine milking, are associated with inherent methodology errors. The oxytocin-handmilking technique adopted in Experiment I often is a convenient method to use but assumes that exogenous hormone administration closely matches the normal process evoked by suckling. Evidence however suggests that, with the doseages commonly employed, exogenous oxytocin administration results in supernormal emptying of the udder (McCance 1959; Coombe et al. 1960) which in itself may promote milk secretion (Linzell and Peaker 1971b). As well, the extrapolation of milk production measured over a short period, usually of 1 - 3 h duration, to an estimate of 24 h milk yield assumes that diurnal variation in milk secretion is insignificant, although some evidence points to the contrary. At best then the oxytocin technique may be useful in measuring the maximum potential of the mammary gland to produce milk over a short period of time but may not be a good estimate of the normal daily production of the animal.

While the weigh-suckle-weigh technique used in Experiment II, theoretically at least, measures the amount of milk ingested by the lamb, it too is subject to inherent errors, the primary one being the inaccuracy of milk intake measurement, particularly as the lamb increases in size. Furthermore, although the normal suckling interval of an undisturbed lamb has been reported to be 2.5 - 3 h Ricordeau et al.(1960, cited by Gardner and Hogue 1964), many experiments have adopted a much longer suckling interval (4 - 6 h) as a matter of convenience. If an excessive period of time is left between sucklings, the lamb may have difficulty in withdrawing all of the accumulated milk (Coombe et al. 1960). Finally, if the conditions associated with the milk yield procedure cause stress and the endogenous release of adrenaline and noradrenaline, evidence suggests that the milk ejection response may be hindered by a partial blocking of oxytocin release from the posterior pituitary (Cross 1955), direct inhibition of the action of oxytocin on mammary myoepithelial cells (Chan 1965) or by the constriction of mammary blood vessels and increased resistance of the mammary ducts (Hebb and Linzell 1951). Unless these errors can be avoided, the weigh-suckle-weigh technique of measuring milk production may result in an underestimation of the daily milk production of the ewe.

In the single published study that has employed both the weigh-suckle-weigh and oxytocin techniques to measure milk yield in the same ewes, the weigh-suckle-weigh procedure consistently gave lower results (Coombe et al. 1960). This study may be criticized however since a 5 h suckling interval was used during the estimation of milk production on the basis of the lamb's intake and a 2 h accumulation of milk was used to estimate 24 h milk yield with the oxytocin technique. As discussed previously, these factors may bias the results obtained using these two procedures.

Previous studies have shown that milk secretion can be depressed in cows by low temperatures experienced during winter conditions (MacDonald and Bell 1958) and in the laboratory, by local cooling of the udder (Holmes 1971). Extensive metabolic studies with goats under laboratory conditions also have proven that acute cold exposure can detrimentally affect milk secretion (Clarke et al. 1976; Thompson and Thomson 1977; Thomson et al. 1979, 1980; Faulkner et al. 1980).

Some of the factors that are thought to be involved in a cold-induced depression of milk yield include:

1. Reduction in Mammary Blood Flow: blood flow to the udder has been implicated as a possible limiting factor to the level of milk production since it can influence both the availability of substrates and the hormonal balance of the mammary gland. Thompson and Thomson (1977) however discovered, by using two levels of acute cold treatment, resulting in either a 18 % or 46 % increase in the metabolic rate of lactating goats, that milk secretion

was depressed by both cold treatments but mammary blood flow fell only during the more severe cold exposure. They concluded that the reduction in milk secretion observed in a mildly cold environment was related to factors other than mammary blood flow, although in a colder environment, blood supply to the udder may be one of the mechanisms that limits milk secretion (Thompson and Thomson 1977).

2. Dehydration: Goat's milk consists of 900 ml water/l, consequently the inavailability of water itself may limit the level of milk production. During a 24 h period of cold treatment, resulting in approximately a 25 % increase in metabolic rate, Thomson et al. (1980) studied the fluid balance of lactating goats. Although water intake was immediately reduced, a decrease in urine output was not noted for several hours. Milk secretion was also depressed by the low temperature, possibly due to the higher osmolality of the plasma, thus retarding the osmotic movement of water from the blood to milk. Although a change in fluid balance of the lactating goat was correlated with depressed milk secretion during cold exposure, Thomson et al. (1980) concluded that this factor could account for only 12 % of the observed decrease in milk fluid volume.
3. Reduced Substrate Supply: Since lactose is the main osmolar component of milk and water moves from the bloodstream into milk through osmosis, lactose secretion

may regulate total milk production. Normally milk lactose is derived only from plasma glucose (Hardwick et al. 1963); a change in the uptake of glucose by the mammary gland could therefore alter the rate of lactose formation and, consequently, of milk secretion. This possibility was examined by Faulkner et al.(1980). During a 24 h exposure of lactating goats to a cold environment, resulting in a 50 % increase in metabolic rate, glucose uptake by the mammary gland, lactose secretion and milk yield all decreased by 69 %. From this experiment it was reasoned that a reduced secretion of lactose was a major factor contributing to a depression in milk secretion in cold-stressed goats.

Other factors which have been postulated to influence the milk producing capacity of the cold-stressed goat but have not been extensively examined include:

1. Energy Balance: In the goat, Thompson and Thomson (1977) suggested that the increased energy losses to the environment during acute cold exposure were only partially offset by a reduction of energy lost in the milk. The net energy deficit of the cold lactating goat therefore, may, by itself, reduce milk secretion.
2. Hormones: In addition to the endocrine changes already mentioned, cold exposure has been reported to increase corticosteroid secretion (Tanche 1976; Alexander, 1979) and is believed to result in lower circulating levels of prolactin, at least in man (Mills and Robertshaw 1981),

heifers (Tucker and Wettemann 1976) and steers (Smith et al. 1977), acting perhaps by blocking prolactin release from the anterior pituitary (Tucker and Wettemann 1976).

The effect of hormonal changes induced by cold exposure on the capacity of an animal to produce milk has received little attention nor has the endocrine balance necessary to maintain milk secretion at a high level during established lactation been clearly defined. Studies of the hormonal requirements for the maintenance of lactation in ruminant species have concentrated on the dairy cow. In this species, Tucker (1979) stated that prolactin, growth hormone (GH), ACTH or glucocorticoids, TSH or thyroid hormones, insulin and parathyroid hormone are necessary for the maintenance of a high level of milk secretion and oxytocin is necessary for milk removal. There is evidence however of wide variation between unrelated species of animals in terms of the hormonal balance necessary to maintain lactation (DeLouis et al. 1980) and perhaps even of variation between different types of ruminant animals: in the cow and goat, administration of the ergot alkaloid, ergocryptine, to block prolactin release, has little effect on milk yield (see review by Bauman and Currie 1980) but it has a potent antigalactopoietic effect in the ewe, which can be reversed by the administration of exogenous prolactin (Hooley et al. 1978).

In further work changes in plasma levels of hormones were correlated with changes in the milk yield of the cow. Hart et al. (1978) compared plasma hormone profiles of high and low yielding cows and determined that plasma levels of GH were increased approximately 3-fold in high producing cows. In the low yielding cow, insulin levels were found to be consistently higher and plasma T_4 levels also tended to be elevated. Plasma prolactin concentration did not differ between the two groups (Hart et al. 1978). Additional research by the same group has found high positive correlations between a change in milk yield and a change in the ratio of plasma GH to T_4 and to insulin, and a negative correlation between shifts in milk production and plasma T_4 levels in lactating dairy cows (Hart et al. 1979). Although this research may provide some insight into the endocrine mechanisms associated with lactation in the cow, the results may not necessarily extend to the lactating ewe.

A study in which the effect of cold stress during lactation on endocrine metabolism was examined was performed by Faulkner et al. (1980). In the lactating goat, reduced mammary uptake of glucose during cold exposure was associated with an increased circulating level of corticosteroids although no change in insulin concentration was observed. Robertshaw (1981) suggested that the reduction in milk secretion that has been reported to occur in goats during acute cold exposure and in cows during winter conditions may be related to a suppression of prolactin release. As Tucker (1979) however has pointed out, prolactin concentration in the plasma does not appear to be clearly related to milk yield nor does it appear necessary for the maintenance of lactation in these species. It is possible that the inhibition of milk secretion that has been observed in cold-stressed goats and cows is related to a catecholamine-induced inhibition of the milk ejection reflex (Hebb and Linzell 1951; Cross 1955; Chan 1965) or may reflect a supression due to elevated thyroid hormone levels. Logically however, an endocrine-mediated depression of milk secretion in a cold environment must be related to a complex of hormones rather than to the actions of any single one.

In the current experiments, milk production in the ewe was not consistently affected by cold exposure. These results clearly contradict those obtained previously with cold-stressed goats. Although the cause of the discrepancy between the present studies and previously published reports is unknown, certain factors may have contributed to the results:

1. Severity of Cold Stress: The cold treatment used in the current experiments resulted in a 20 - 55 % increase in metabolic rate. This severity of cold results in a significant decrease in milk production in goats even

after a short period of treatment (Clarke et al. 1976; Thompson and Thomson 1977; Thomson et al. 1979,1980; Faulkner et al. 1980). It is possible that milk production in the ewe is more resistant to a low environmental temperature than in other animals.

2. Duration of Cold Exposure: All of the laboratory studies that have examined the influence of cold stress on lactation have used treatment periods of 24 - 48 h duration. It is possible that a low temperature-milk secretion interaction is of short duration and is not expressed during more prolonged periods of cold exposure. This hypothesis is not supported by evidence that in dairy cows, milk production measured in field trials decreases during winter conditions (see review by Johnson 1976), although several factors other than a low environmental temperature may have contributed to these results.

3. Behavior: Gonyou et al.(1979) demonstrated that cold-stressed cattle spend a larger percentage of their time lying down than do cattle in a thermoneutral environment. If the CE ewes in the present experiments laid down for a large portion of each day, the mammary gland may have been sufficiently insulated to reduce the effect of low temperature on mammary metabolism. Evidence presented in Chapter III supports that mammary blood flow and udder surface temperature are not significantly affected by a low ambient temperature when

the ewe is in a lying position although both parameters decrease markedly when the animal is standing.

4. Hormones: It is known that the hormonal balance necessary for the maintenance of a high level of lactation varies widely between species (DeLouis et al. 1980). Alterations in endocrine metabolism that occur during cold exposure may therefore have variable effects on lactation, depending upon the species of animal examined.
5. Energy Balance: From the calculations of heat production, energy output in the milk and metabolizable energy intake it is probable that the CE ewes were in a situation of net energy deficit during the majority of the experiment. The magnitude of energy deficit however might have been overestimated since changes in behavior as a result of experimental manipulations may have induced an increased level of heat production during the periods of measurement (ie. the ewes may have spent a greater proportion of time standing during periods of measurement of Hp than they would normally). The magnitude of the animal's energy deficit may indeed be a critical factor in determining the level of lactation.
6. Influence of Suckling Young: Within the limits set by genetic capacity, milk production in the ewe is related to both food intake and the number of young. Ewes suckling twins generally produce 20 - 40 % more milk than those suckling single lambs (NRC 1975), possibly

due to the hormonal effects induced by a greater frequency and/or intensity of suckling. Since, in the present studies, the lambs as well as the ewes were housed at a low ambient temperature, the cold environment may have led to a change in suckling behavior possibly resulting in a greater level of milk production. Other trials that have maintained lactating animals in the cold have used hand- or machine-milked goats or cows; in these experiments, the influence of the suckling young on milk production would not have been present.

7. Technique of Milk Yield Measurement: Since the animals used in the present studies were not accustomed to regular milking prior to the start of the experiments, the techniques used to measure milk production may not have truly reflected the normal daily milk secretion and could perhaps have masked any treatment effect.

Additional research is required to further examine the lack of effect of chronic cold exposure on milk production in the ewe.

Milk Composition

In the ewes housed at a thermoneutral temperature, protein concentration of the milk averaged 5.09 % in Experiment I and 4.80 % in Experiment II. Published values for protein concentration in ewes' milk have ranged from 4.11 to 5.50 % (Barnicoat et al. 1957; Gardner and Hogue 1964; Williams et al. 1976; Davis et al. 1978;

Torres-Hernandez and Hohenboken 1979). Average values of percent lactose were calculated to be 5.60 and 5.46 in Experiment I and II respectively, and are within the range of previously reported data (Barnicoat et al. 1957; Gardner and Hogue 1964; Williams et al. 1976; Davis et al. 1978; Torres-Hernandez and Hohenboken 1979). Fat formed the largest component of the milk solids, with an average concentration in milk of 7.41 % in Experiment I and 7.90 % in Experiment II. While Barnicoat et al. (1957) determined that percent fat in ewe's milk averaged 5.5 %, more commonly, values of 6-8.5 % have been reported (Gardner and Hogue 1964; Williams et al. 1976; Davis et al. 1978; Torres-Hernandez and Hohenboken 1979).

Cold exposure tended to result in an increase in percent milk fat, which became statistically significant as the duration of cold exposure increased. Percent lactose was not significantly altered in either experiment but in Experiment II, percent milk protein tended to be higher as a result of cold exposure.

Previous studies that have examined the effect of acute cold stress on milk composition of the goat have reported that total lactose yield decreases during cold exposure (Clarke et al. 1976; Faulkner et al. 1980) but is related to a significant reduction in total milk yield rather than in lactose concentration (Faulkner et al. 1980). Clarke et al. (1976) found that milk protein and fat yields were significantly depressed after 24 h of cold exposure but showed some recovery after 48 h of treatment, despite a continued depression in the rate of milk secretion. This data may indicate then that fat and protein concentrations were increased in the milk collected after 48 h of thermal treatment. Thomson et al. (1979) also determined that total milk fat secretion in the cold-stressed goat was maintained although milk secretion was depressed. Additionally, several studies have determined that milk butterfat percent increases when cows are exposed to a low ambient temperature (see review by Johnson 1976), although MacDonald and Bell (1958) were unable to detect any cold-induced change in percent milk butterfat or protein in ad-lib fed cows housed outdoors during Canadian winters.

Milk Fatty Acid Composition

Fatty acids secreted in milk are derived either from de novo synthesis in the mammary gland or from plasma triglycerides and free fatty acids; the relative contribution of each source of fatty acid precursors is largely dependent on species and physiological status (Falconer 1980). In ruminants, milk fatty acids ranging from 8 to 14 carbons in length are normally synthesized de novo in the mammary gland from carbon skeletons provided by plasma β-hydroxybutyrate and acetate (Linzell et al. 1967), whereas fatty acids longer than 16 carbons in length normally are derived from plasma free fatty acid and triglyceride pools (Annison et al. 1967). Glucose carbon cannot be directly used for fatty acid synthesis in the ruminant mammary gland (see review by Linzell and Peaker 1971a) however mammary glucose oxidation via the pentose cycle provides an important source of reducing equivalents necessary for fatty acid synthesis (Bauman and Davis 1974).

Studies performed with ruminants housed in thermoneutral environments have uniformly reported that long chain fatty acids, primarily palmitic (C16:0) and oleic (C18:1) acids, form the largest proportion of the total fatty acids secreted in milk. In the studies reported by Thomson et al. (1979) and Chaiyabutr et al. (1980a), respectively, palmitate and oleate together formed 49.9 % and 43.2 % of the total milk fatty acid secretion in goat's milk. In the ewe, Thompson and Goode (1981) found that these two fatty acids constituted approximately 63.2 % of the total milk fatty acids and, in the present study, 51.3 % of the total fatty acid secretion in the CON ewes was attributable to palmitic and oleic acids. Short chain fatty acids (less than 16 carbons in length) were found to comprise 32.8 % (Thomson et al. 1979) and 39.9 % (Chaiyabutr et al. 1980a) of the total fatty acid secretion in goats, and 27.1 % in ewes (Thompson and Goode 1981), again corresponding well with the average value of 32.9 % determined in the current study.

In the present experiment, prolonged cold exposure resulted in a relative decrease in mammary secretion of all of the short chain fatty acids, but an increase in long chain fatty acid secretion, reflecting a tendency towards elevated secretion of stearate (C18:0) and the cis form of oleate (C18:1). Clarke et al. (1976) and Thomson et al. (1979) have also determined that a short term exposure (48 h) of lactating goats to a moderate cold stress, sufficient to increase heat production by 28 - 46 %, decreased milk secretion of short chain fatty acids but increased that of long chain fatty acids, especially of oleic acid.

Modification of mammary fatty acid metabolism during acute cold exposure in the lactating goat has been examined using radioactive isotopes and has been found to be caused by a change in both the rate of supply and of mammary uptake of fatty acid precursors. A shift away from short chain fatty acid secretion in the milk of the cold-stressed goat can be related to a decreased supply and uptake of acetate (Thomson et al. 1979), β-hydroxybutyrate Faulkner et al.(unpublished, cited by Faulkner et al. 1980) and a reduced mammary uptake of glucose (Faulkner et al. 1980), resulting in a decreased supply of carbon skeletons and of reducing equivalents necessary for de novo fatty acid synthesis. On the other hand, free fatty acid supply and mammary uptake are increased during cold exposure (Thomson et al. 1979), supporting an increased release of long chain fatty acids in milk.

Organ Weights

Prolonged exposure of the lactating ewe to a cold environment was not accompanied by any change in weight relative to total bodyweight of the abdominal (gastro-intestinal tract, kidneys) or thoracic (lungs) organs examined. Other studies with growing pigs have also failed to demonstrate any cold-induced change in digestive organ weight (Sugahara et al. 1970; Hacker et al. 1973; Brown et al. 1976), although measurements performed by Graham et al. (1982) and Schaefer (1979) did suggest that certain portions of the gastro-intestinal tract were hypertrophied in chronically cold-stressed wethers. The results in the latter two experiments however may have been related to treatment differences in feed intake rather than to differences in ambient temperature.

In comparison to other studies of organ weights performed in this laboratory with wethers (Schaefer 1979; Graham et al. 1982) and young lambs (Chapter IV), the alimentary tract of the lactating ewe was more extensively developed, although the results are confounded by nutritional factors. Expressed in terms of weight per unit of bodyweight, the reticulo-rumen, omasum, abomasum and large intestine were found to be larger in the lactating ewe than in either the lamb or wether while small intestine weight was largest in the young lamb, followed in decreasing order by the lactating ewe and wether. Fell et al. (1964), Tulloh (1966) and Chatwin et al. (1969) have also reported a hypertrophy of the digestive tract during lactation in the ewe, cow and rat, respectively, that may be related to an increased food intake rather than a direct influence of lactation *per se*. In the rat at least, increased gastro-intestinal tract weight during lactation is accompanied by an increased total blood flow (ml/min) and proportion of total cardiac output (Chatwin et al. 1969; Hanwell and Linzell 1973). Presumably these changes in digestive tract development and blood supply are important adjustments to a higher level of food intake and an increased requirement for nutrient absorption to support mammary metabolism.

Although other organ weights were not affected by cold exposure, mammary gland weight, expressed as a percentage of bodyweight, was reduced in the CE ewes. Further dissection of the mammary gland would suggest that the reduction in mammary weight was due primarily to a decreased secretory tissue mass (Chapter III). Since, in the ewe, the number of secretory cells in the udder virtually does not increase after parturition (Anderson 1975), a smaller mammary gland may be reflective of a reduced degree of hypertrophy of the secretory cells during lactation. The cause of a lack of mammary development in a cold environment remains to be identified.

The evidence of a larger adrenal gland size suggests that adrenal function was increased during cold exposure. This hypothesis is supported by the observed increases in the circulating levels of catecholamines in the CE ewes. Similarly, the trend towards thyroid gland hypertrophy in the cold-stressed ewes agrees well with the increased plasma levels of T_3 and T_4 observed.

Efficiency of Milk Production

Linzell (1972) postulated that milk yield per unit mammary tissue is similar in all species. Data collected from various ruminant species would support this hypothesis. Quoted values of 1.90, 1.75 and 1.66 g milk/g mammary tissue during established lactation in the cow, dairy goat and non-dairy goat (Linzell 1972) respectively, compare favorably with the value of 1.84 g milk/g mammary tissue reported for the ewe (Davis et al. 1978). As the data in the current experiment was collected from ewes nearing the end of lactation, a value of 1.22 g milk/g mammary tissue in the CON ewes is in keeping with previous reports.

Since other studies have not directly assessed secretory tissue mass, values of milk yield/unit secretory tissue have not been reported. On the basis of dissection studies, secretory tissue forms approximately 84 % of the total mammary weight in lactating dairy goats (Linzell 1960) and 87 % of the total udder weight of the ewe at mid-lactation (Thompson 1980). After adjustment of the data collected by Linzell (1972) and Davis et al. (1978), milk yield (g)/g secretory tissue would approximate 2.08 and 2.11 in the dairy goat and ewe respectively. In Experiment II, mean milk production (g)/g secretory tissue in the control ewes was calculated to be 1.84 and therefore is lower than other estimates. In these animals however, secretory tissue formed only 66 % of the total mammary weight (Chapter III), further supporting the conclusion that the ewes were in declining lactation at the time of measurement.

The ratio of blood flow:milk yield has been suggested to be an appropriate measure of mammary efficiency since this ratio varies inversely with the rate of milk secretion (Linzell 1960). Previous studies have reported average blood flow:milk yield values of 507 (Peeters et al. 1979), 457 (Bickerstaffe et al. 1974) and 390 (Paterson and Linzell 1974) for the cow and 689 (Thompson and Thomson 1977), 523 (Thomson et al. 1979), and 438 (Chaiyabutr et al. 1980b) for the goat housed in a thermoneutral environment. Much of the variation within a species may reflect differences in the stage of lactation; by measuring the changes in the ratio of blood flow:milk yield over the course of lactation in the dairy goat, Linzell (1974) demonstrated that while the ratio averaged 460:1 at peak lactation, during the major portion of lactation, values averaged 500:1 and increased to 700 - 1000:1 as milk yield fell. In the ewe, the average value of 870:1 for blood flow:milk yield reported by Davis and Bickerstaffe (1978) is higher than the mean value of 664:1 determined in the present experiment. It is probable that the ratio of blood flow:milk yield was underestimated in the current study since milk yield was determined 2 wk prior to measurement of mammary blood flow. The apparent relative inefficiency of milk secretion in the ewe compared to other ruminants has been attributed to the greater total solids content of ewe's milk (Davis and Bickerstaffe 1978).

Mild and moderate cold stress, respectively, was found to result in a 26 % and 17 % decrease in mammary efficiency as measured by blood flow:milk yield in the study performed by Thomson et al. (1979) and a 49 % and 84 % decrease in a similar study reported by Thompson and Thomson (1977). Chaiyabutr et al. (1980b) also found that 48 h starvation in the goat caused the ratio of mammary blood flow:milk yield to rise from a pre-treatment value of 438:1 to 483:1. In the present study however, mammary efficiency expressed as g milk/g mammary tissue or g secretory tissue and as blood flow:milk yield would suggest that the efficiency of milk synthesis was greater in the chronically cold-exposed ewes than in the control animals.

In summary, the data collected in the present experiments did not reveal any detrimental effect of prolonged cold exposure on the lactational performance of the ewe but in fact suggested that the efficiency of milk production may have been increased as a result of a low ambient temperature. Since these results contradict those obtained with acutely cold stressed goats, further work will be required to isolate the factors responsible for the observed responses.

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III. Section II

Factors Affecting Mammary Blood Flow in the Lactating Ewe With Particular Reference to the Effects of Acute and Chronic Cold Exposure

A. Abstract

Mammary blood flow was measured in lactating ewes using an electromagnetic blood flow probe or radionuclide labelled microspheres. Continuous recording of blood flow through the mammary artery revealed that changes in posture, feeding, minor stresses and suckling caused blood flow to decrease. During a period of acute cold exposure (4 h) mammary blood flow, trunk, udder and extremity surface temperatures decreased markedly when the animal was standing; heart rate increased but rectal temperature remained relatively unchanged. Lying down effectively insulated the mammary gland from low ambient temperatures as indicated by mammary blood flow and udder surface temperature.

After a period of prolonged cold exposure (0 °C, 8 wk), udder size and mammary blood flow tended to be lower in comparison to values obtained from ewes housed for the same period at 21 °C. Cold exposure also affected blood flow distribution: as a proportion of total mammary blood supply, blood flow to the secretory tissue was reduced by approximately the same amount as connective tissue blood flow was increased. Microspheres injected before and after

suckling revealed that suckling caused a decreased blood flow to the mammary gland in 4 of the 5 animals but did not significantly alter blood flow distribution. Blood flow was increased to the popliteal fat depot ($P<0.01$) and tended to be higher to the gastrocnemius and semimembranosus muscles ($P>0.10$) but was decreased to the skin of the thigh ($P<0.05$) and lower leg ($P<0.10$) as a result of cold exposure.

B. Introduction

Under certain conditions, restricted circulation to the mammary gland of the lactating animal may influence the milk producing capacity of the gland since the synthesis of milk constituents is dependent upon the uptake of precursors from the bloodstream (Linzell 1974). Knowledge of the factors that influence and control mammary blood flow (MBF) would therefore aid in our understanding of the mechanisms and limitations to the process of milk synthesis.

Numerous techniques have been used to examine MBF in both laboratory and domestic livestock species (for a review of methods see Reynolds et al. 1968; Linzell 1974). Many studies have attempted to define variations in MBF that occur under normal physiological conditions (Linzell 1960; Chatwin et al. 1969; Hanwell and Linzell 1973a,b; Mao and Caruolo 1973; Burd et al. 1975; Davis and Bickerstaffe 1978; Fleet and Peaker 1978; Peeters et al 1979; Burvenich 1979; Davis et al. 1979; Chaiyabutr et al. 1980a,b) and from the administration of exogenous hormones and vasoactive substances (Hebb and Linzell 1951; Houvenaghel 1970; Houvenaghel and Peeters 1971,1972; Dhondt et al. 1973,1977; Houvenaghel et al. 1973; Burd et al. 1978a,b; Burvenich 1980; Hart et al. 1980; Henderson and Peaker 1980).

The influence of the combined stress imposed by a cold environment and lactation on blood flow to the mammary gland has received little attention in the literature. General circulatory changes that occur as a result of cold exposure are well documented: increased heart rate, cardiac output and a redistribution of blood flow help the animal to maintain a state of thermal equilibrium despite unfavorable environmental conditions (see reviews by Thauer 1965; Webster 1974; Thompson 1977; Alexander 1979). During lactation, increased cardiac output and blood flow to the liver, gastro-intestinal tract and mammary gland may be necessary adjustments to support the process of milk formation (Chatwin et al. 1969; Hanwell and Linzell 1973a). Under conditions of cold stress and lactation the circulatory system therefore must function to:

1. conserve heat by reducing peripheral flow
2. support the increased rate of metabolism in thermogenic tissues by supplying substrates and removing metabolic endproducts and
3. provide the necessary substrates required for milk synthesis in the mammary gland.

Studies performed by Thompson and Thomson (1977), Thomson et al. (1979) and Faulkner et al. (1980) have shown that the exposure of lactating dairy goats to a moderate cold stress for 24 - 48 h generally results in a decrease in total MBF that can partially account for a concurrent drop in milk production. Distribution of blood flow within the mammary gland was not determined in these studies. Although the results are limited, the influence of cold on blood flow to the mammary gland seemed to diminish over the two days of cold exposure (Thompson and Thomson 1979). No studies to date have examined MBF in a lactating ruminant over a more prolonged period of cold exposure.

The present experiments were designed to examine both the influence of normal daily routines and of cold stress on MBF in lactating ewes. In the first experiment, the changes in MBF as a result of diurnal variation, feeding, suckling, posture and acute cold stress were measured and compared. A second experiment was performed to examine the influence of prolonged cold stress during lactation on mammary blood flow and distribution in the ewe.

C. Materials and Methods

Experiment I

Animals

Two crossbred Suffolk ewes, averaging 44.4±3.5 kg in weight were used in this study. Both ewes were nursing single lambs. Each ewe-lamb pair was housed in a metabolic crate sufficiently large to permit unrestricted movement (0.9x1.8 m). 1000 g of a pelleted barley-alfalfa mixture (38 % barley, 62 % alfalfa) was fed twice daily (0800 and 1600 h) to each ewe. Free access to water and cobalt-iodized salt was also provided.

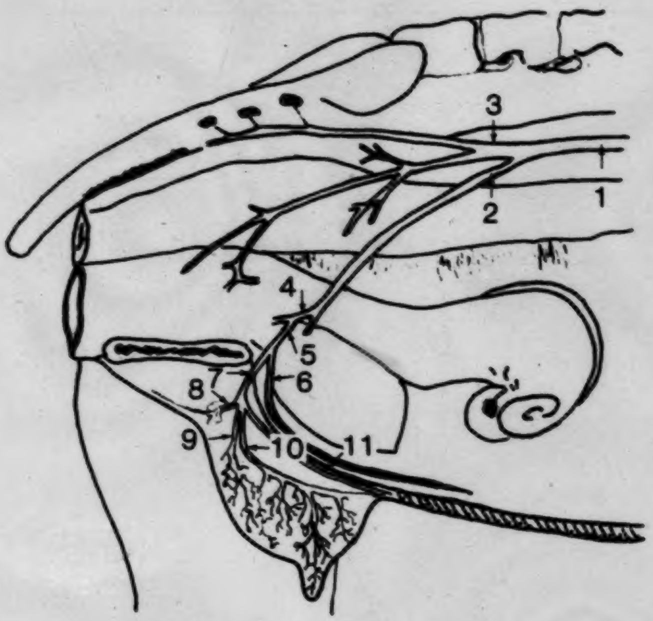
Arterial Supply to the Mammary Gland of the Ewe

Blood flow to each udder half is derived from the external pudic artery which is a major branch of the external iliac artery. The external pudic artery passes through the inguinal canal and divides into four vessels (Figure III.1): two vessels supply the mammary gland itself (mammary artery and caudal mammary artery), one vessel supplies blood to the supramammary lymph nodes and another to the superficial portion of the abdomen (Tanudimadja et al. 1968).

Surgery

During the initial stages of lactation, each ewe was surgically fitted with an electromagnetic blood flow probe on the right mammary artery. The vessel was located under general anaesthesia according to the

Figure III.1 Arterial Supply to the Hindquarters and Mammary Gland of the Ewe.



1. Dorsal Aorta
2. External Iliac Artery
3. Internal Iliac Artery
4. Deep Femoral Artery
5. Pudendo-Epigastric Trunk
6. Caudal Deep Epigastric Artery
7. External Pudic Artery
8. Branch to the Supramammary Lymph Nodes
9. Branch to the Caudal Portion of the Mammary Gland
10. Mammary Artery
11. Caudal Superficial Epigastric Artery (Cranial Mammary Artery)

procedure of Burd et al. (1975). An incision was made along the lateral border of the cranial portion of the right udder half and the dissection deepened through the subcutaneous layers and mammary fascia until the prominent mammary vein (external pudic) was identified. The mammary artery, lying in close proximity to the vein, was carefully freed from surrounding fascia over a 2 cm length cephalad to its first point of arborization. The head of a flow probe (Carolina Medical Electronics; 16 mm internal circumference) was slipped over this section of the artery and secured in place. The lead wires of the probe were sutured to the surrounding fascia and passed subcutaneously to a point on the right flank, 6 -10 cm ventral to the spine, where they were exteriorized. Sufficient slack was left in the lead wires to permit the probe head to ride freely on the artery during movement of the animal. MBF measurements were not commenced until 1 -2 wk after surgery to allow the animal to recover and the probe to become securely attached to the vessel.

Experimental Procedures

Blood flow measurements were obtained from each ewe in both a thermoneutral and cold environment. The thermoneutral trials (21 °C) were performed over a 12 (1000 - 2200 h) or 24 (1000 - 1000 h) h period. During the trial the ewe remained in her crate but was tethered with a neck collar to restrict movement. Feed and water were provided as described earlier. Throughout the experimental period the lamb was separated from its dam by placing it in a wire cage within its dam's pen and allowed to suckle at 3 h intervals. This procedure permitted visual contact during separation of the ewe and lamb, which was necessary to minimize maternal stress, but did not disrupt the normal suckling pattern of the lamb.

Mean and pulsatile MBF was continuously recorded by means of a square wave electromagnetic flowmeter (Carolina Medical Electronics) connected to a physiograph recorder (Beckman R-612 Dynograph). Several times during the recording period, zero blood flow was electronically calibrated to check for baseline drift. An event recorder was used to determine the occurence and duration of changes in posture (standing/lying).

The influence of acute cold stress on MBF was determined using a temperature-controlled chamber. The ewe was shorn to a fleece depth of approximately 1 cm 1 - 3 days prior to measurement and moved into a large metabolic crate (1.2x1.2 m) within the chamber at least 12 h before the commencement of the trial. As during the thermoneutral trial, the ewe was tethered with a neck collar and the lamb confined to a wire cage within its dam's pen. Feed, water and suckling procedures were as those previously described.

Prior to 0800 h on the day of the experiment, physiological monitoring equipment was attached to the animal and the recording cables extended through portholes in the chamber wall. Twelve thermocouple leads were also passed through the porthole to monitor ambient temperature within the chamber.

A continuous trace of mean and pulsatile MBF was attained as described for the thermoneutral trial. Recording cables from heart rate and electromyograph electrodes, an event recorder and a rectal probe, inserted 15 cm into the rectum, were also attached to the dynograph recorder, located outside of the chamber. Surface temperatures of the trunk (right hip, left abdomen, right abdomen, left forearm, mid-dorsal region of the rump), extremities (right ear, lateral surface of the left front and hind pasterns) and udder regions (cranial and caudal portions of the mammary gland, medial surface of the left thigh) were attained by gluing adhesive covered copper-constantan thermocouples to the skin. A total of 24 thermocouples (12 on the animal, 12 recording chamber temperature) were attached to a 24 channel temperature recorder (Honeywell Electronik-15) which printed one channel per 18 sec.

Basal recordings of all parameters monitored were attained over a 2 h period (0800 - 1000 h). At 1000 h the temperature of the chamber was reduced to 10 °C. Chamber temperature was progressively decreased by 5 °C at 45 min intervals to a final temperature of -15 °C (1045 - 1345 h). At 1415 h the coolers were turned off and physiological recordings continued as the chamber temperature rose (1415 - 1630 h).

Experiment II

Animals

The selection and management of the animals has been previously described for Experiment II, Chapter II. Briefly, 10 ewes nursing single lambs were selected shortly after parturtion and assigned to one of two treatment groups. The control (CON) ewes were housed at a temperature of 21±1 °C for 56 days (wk 2 - 10 of lactation) while the cold-exposed (CE) ewes were kept for the same period of time at an ambient temperature of 0±1 °C. At the end of the exposure period MBF was measured using radionuclide labelled microspheres.

Animal Preparation

One to three days prior to measurement of blood flow to the right half of the mammary gland, microsphere injection and arterial sampling catheters were surgically established according to the procedure outlined by Thompson (1980). General anaesthesia was induced and maintained with halothane. A polyethylene catheter (ID 0.86 mm, OD 1.27 mm) for injection of microspheres was inserted into the right saphenous artery and the tip advanced towards the dorsal aorta. Simultaneously, a thermocouple was inserted into the left femoral artery. Injection of cold saline into the catheter resulted in the flow of injectate down the femoral artery of the left leg which was recorded by a decrease in blood temperature. The tip of the catheter was gradually withdrawn until the temperature of the blood flowing through the left femoral artery did not decrease in response to injection of cold saline. A suture around the saphenous artery was used to hold the injection catheter in place (Figure III.2).

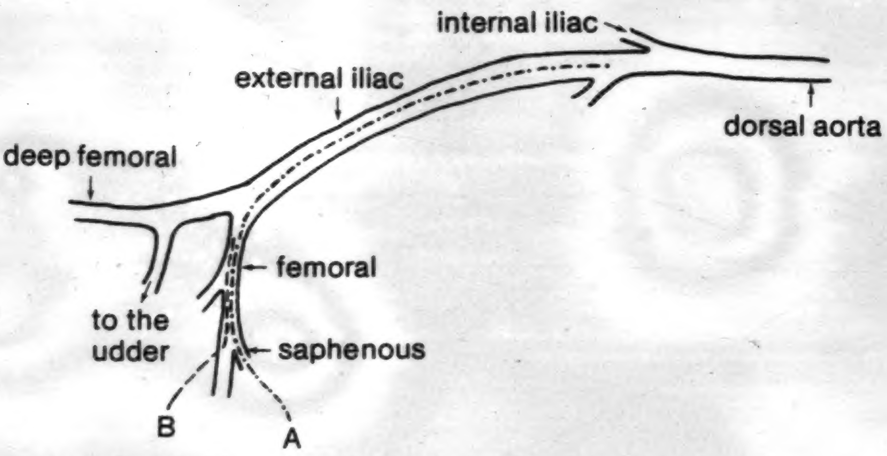
A similar polyethylene catheter was established downstream of the injection site by inserting a catheter into the right femoral artery. The tip of the sampling catheter was advanced 6 cm cephalad before being fixed in position with a purse string suture in the wall of the femoral artery (Figure III.2). Both the sampling and infusion catheters were sutured to the surrounding fascia and passed subutaneously to a point of exteriorization on the right flank of the animal. Catheter placement was verified upon post-mortem examination.

Vessels crossing between udder halves were not ligated since blood flow between the two halves in a lactating ewe has been reported to be insignificant (Thompson 1980).

Blood Flow Measurements

Gamma-ray emitting ^{141}Ce- and ^{95}Nb-labelled microspheres (New England Nuclear, Boston, Mass.),

Figure III.2 Placement of Microsphere Injection and Arterial Sampling Catheters, Experiment II.


internal iliac
external iliac
dorsal aorta
deep femoral
femoral
to the udder
saphenous
B
A

A = injection catheter
B = sampling catheter

15±1.2 um in diameter, were used to measure MBF. This size of sphere is sufficiently small to pass through arteries and arterio-venosus anastomoses but becomes lodged in the capillary bed (Hales 1974). Approximately 11.1 uCi ^{141}Ce (14 dpm/bead) and 18.3 uCi ^{95}Nb (20 dpm/bead) was injected into each animal to ensure that the majority of tissue samples counted would contain at least 400 of each type of microsphere. This number of microspheres has been reported to be necessary for an acceptable level of experimental error (Buckberg et al. 1971).

On the day of the microsphere injections, feed but not water was withheld. 4.5 h prior to injection each lamb was separated from its dam by placing it within a wire cage. One and one-half h later the lamb was permitted to suckle and then replaced in the cage.

Three h following the last period of suckling, each ewe was injected with ^{141}Ce-labelled microspheres, suspended in 2 ml of 10 % dextran solution containing a drop of Tween 80 suspending agent. Due to the number of animals involved, microsphere injections were staggered over a 4 h period. A plastic syringe (3 ml) was used to inject the microspheres over a timed interval of approximately 50 - 60 sec. To prevent any settling of spheres, the syringe was constantly rotated during handling and injection. A T-junction at the end of the catheter permitted flushing of the injection syringe with 10 ml sterile saline. Starting immediately prior to and continuing for 30 sec after the microsphere injection, downstream arterial blood was withdrawn from the right femoral artery (artificial organ, Hales 1973) into a plastic 20 ml syringe by means of a Harvard constant infusion/withdrawal pump (Harvard Apparatus, Mills, M.A.).

Following injection of the ^{141}Ce-labelled microspheres, the lamb was permitted to suckle. Ten minutes after the termination of suckling activity each ewe received an injection of ^{95}Nb-labelled microspheres in a similar manner as previously described. Blood samples obtained from the femoral artery during each microsphere injection were weighed and stored at -40 °C until measured for radioactivity.

Twenty-four h after the injection of microspheres each ewe was stunned and bled to death. Just prior to stunning, the lamb was permitted to suckle so that the udder was relatively free of milk. The mammary gland was removed from the carcass and any milk remaining was carefully blotted dry before total udder weight was determined. The right half of the udder was dissected into its various components and the tissues weighed. Samples of lung, kidney, left udder half, gastrocnemius, semimembranosus, popliteal fat depot and skin from the thigh and lower leg were also obtained. Tissue samples were placed in pre-weighed Beckman polyvials, weighed and stored at -40 °C prior to measurement for radioactivity.

Radionuclide counting was performed using a Beckman Gamma 8000 counter. ^{141}Ce and ^{95}Nb sources were used to determine the energy spectra of the two radioisotopes. The counting windows were set so as to encompass the principal photon peaks of the γ-emitters (0.145 MeV for ^{141}Ce; 0.765 MeV for ^{95}Nb), but minimize the spillover of ^{95}Nb counts into the ^{141}Ce channel. Total counts of each sample were corrected for geometrical (Katz and Blantz 1972) and spillover errors, counting efficiency and time decay.

Statistical Analysis

Treatment differences for each microsphere injection were compared using the Students t-test. Values obtained before and after suckling were compared for the CON and CE groups separately, again using the Students t-test (Steel and Torrie 1980).

D. Results

Experiment I

Detailed information regarding the diurnal variation in MBF is presented in Figures III.3, III.4 and III.5 and Table III.1. MBF values varied considerably, both between and within animals. Part of the variation between the two animals may be related to such factors as stage of lactation, age of the animal, size of the mammary gland and milk producing capacity. Since only two animals were used in this study, these factors were not examined.

From the results obtained during a 12 or 24 h continuous recording of MBF in a thermoneutral environment it is apparent that for both ewes, the largest change in MBF was induced by suckling (Figures III.3, III.4, III.5). Invariably the act of suckling resulted in an immediate decrease in MBF. At the cessation of suckling activity, MBF gradually recovered over a period of 30 - 60 sec to a level similar to that recorded prior to suckling (Figure III.5). During the 3 h interval between suckling periods, MBF remained relatively stable (Figures III.3, III.4).

The effect of posture on MBF was especially evident in Animal A (Figure III.3). Frequently a change in posture resulted in a transient drop in MBF. Blood flow to the mammary gland tended to be highest when the animal was lying down (Table III.1).

Feeding resulted in a decreased blood flow through the mammary artery that persisted for a variable length of time

Figure III.3 Diurnal Variation in Mammary Blood Flow of Animal A 34 Days Post-Partum, Experiment I

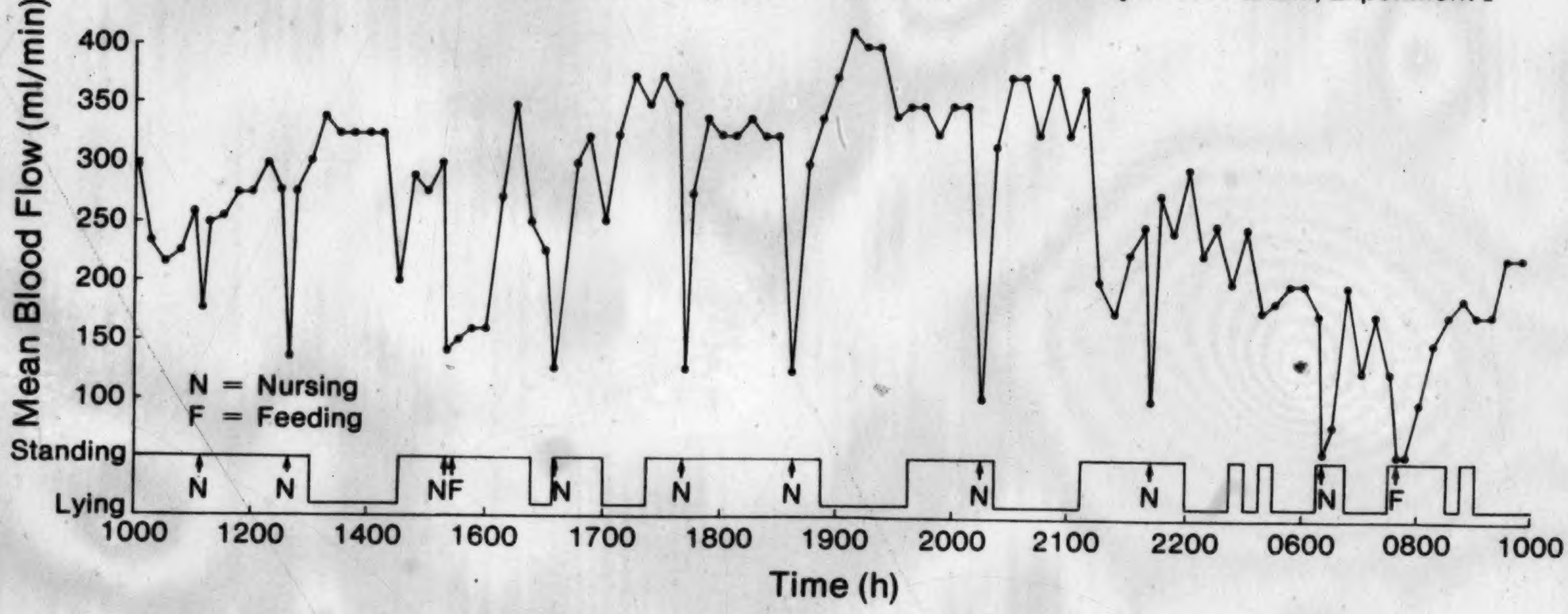


Figure III. 4 Diurnal Variation in Mammary Blood Flow of Animal B 22 Days Post-Partum, Experiment I

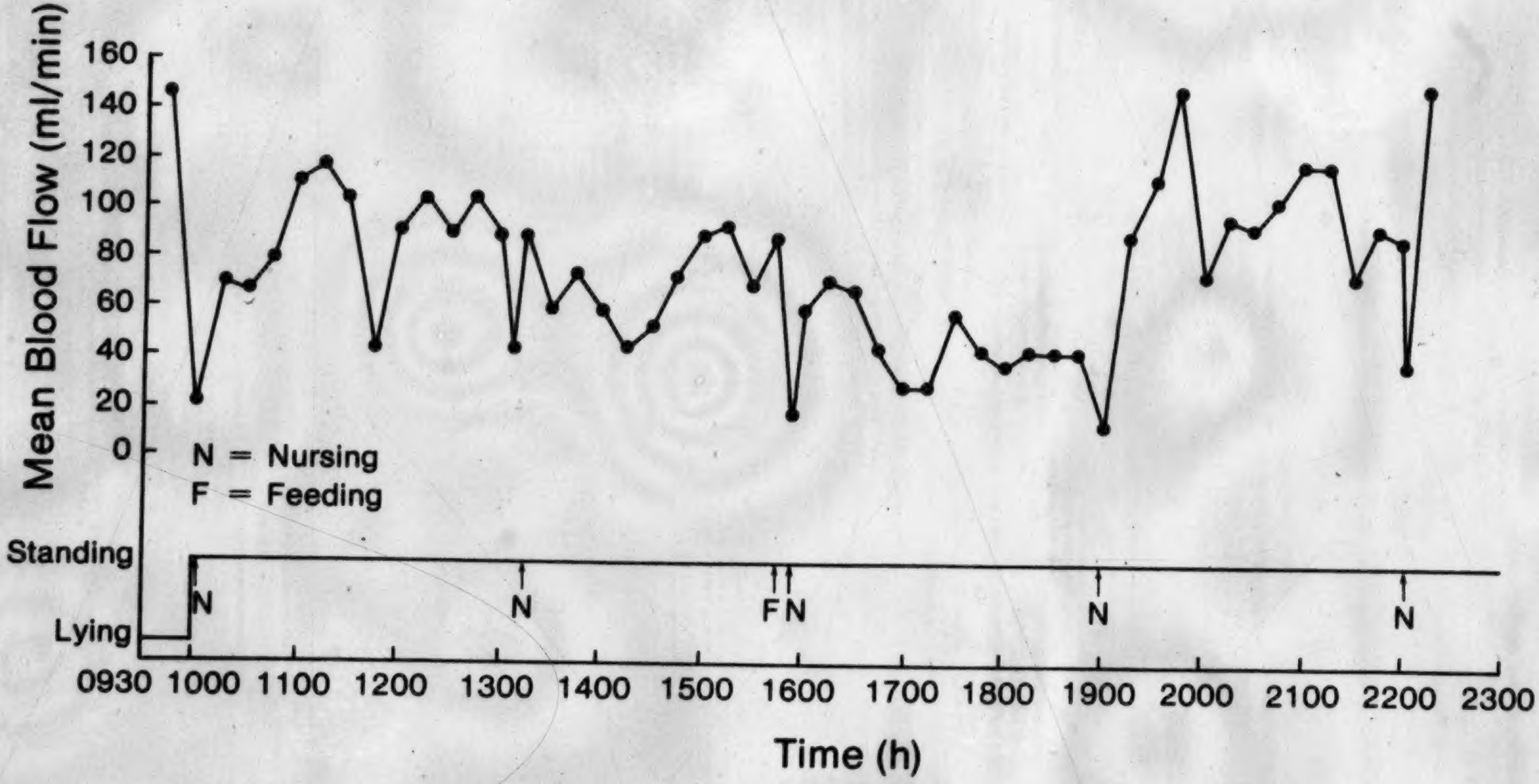


Figure III.5 Influence of Suckling, Feeding and Minor Disturbances on Mammary Blood Flow Measured During Thermoneutral Conditions, Experiment I.

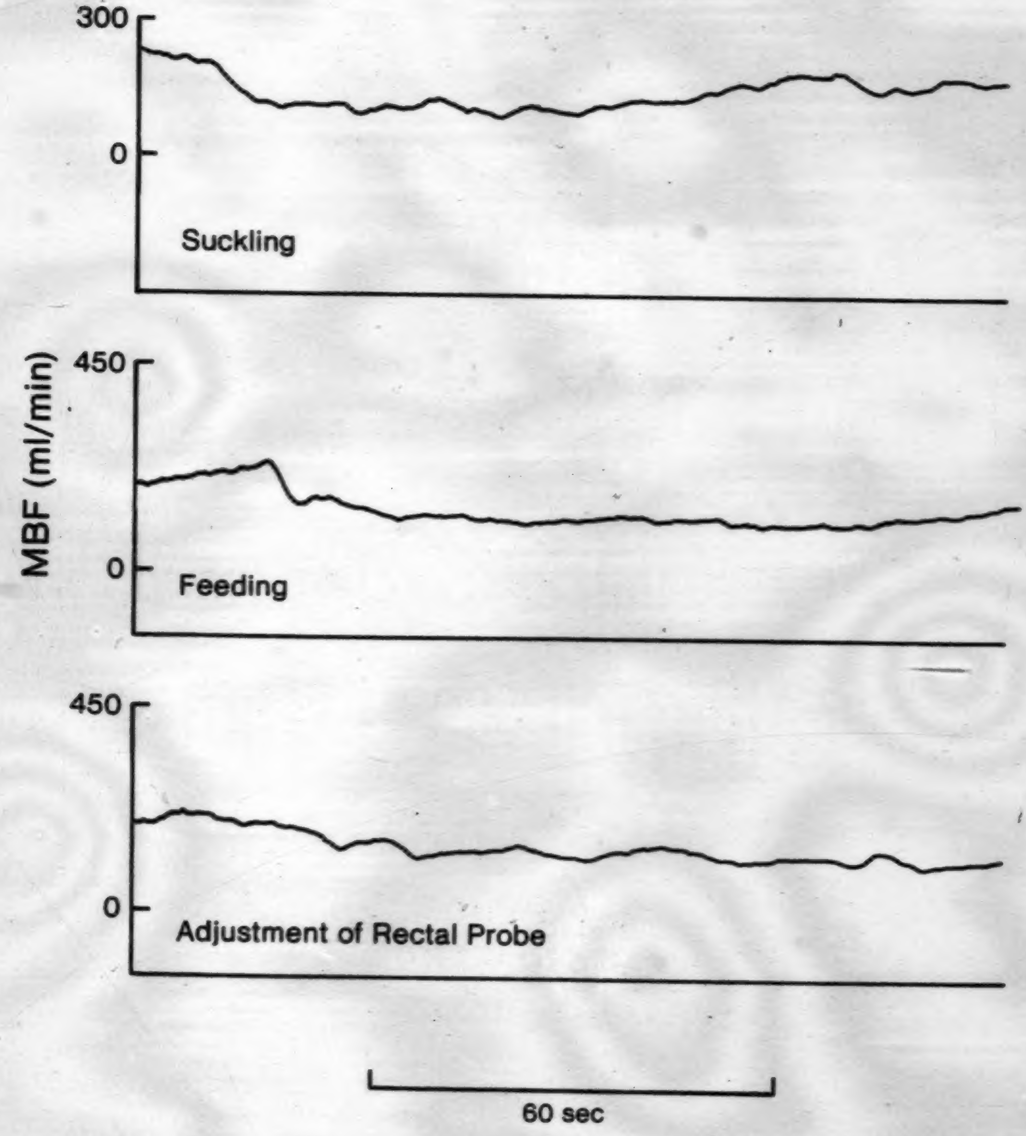


Table III.1 Diurnal Variation in Mammary Blood Flow in Lactating Ewes, Experiment I

	Animal A	Animal B
Mean MBF (ml/min)[1]:		
Standing	258.7±77.7	79.8±28.9
Lying	284.5±77.9	147.0
Nursing	108.3±39.7	28.6±11.7
Feeding	100.0±50.0	68.0
Coefficient of Variation (%)[2]: (period-period)		
Standing	30.0	36.2
Lying	27.4	
Nursing	36.7	35.8
Feeding	50.0	
MBF: % of Mean Standing MBF:		
Standing	100.0	100.0
Lying	110.0	184.0
Nursing	41.9	35.8
Feeding	38.7	85.2

[1] values represent mean±SEM.

[2] coefficient of variation= (SE of MBF x 100)/(mean MBF).

(Figures III.3, III.4, III.5; Table III.1). In both animals a trend towards higher MBF values approximately 4 h following feeding was noted. This tendency was particularly evident in Animal A between the night and morning feedings (Figure III.3).

Minor disturbances (ie. experimenter entering the room, adjustment of equipment) usually resulted in a small decrease in MBF (Figure III.5).

Physiological measurements recorded during a period of acute cold stress are presented in Figures III.6, III.7 (Animal A) and Figures III.8, III.9 (Animal B). Although both animals were exposed to a similar degree and duration of cold stress, each animal employed different strategies to combat the unfavorable environmental conditions. Animal A exhibited few behavioral signs of cold stress; except for a few short periods, this animal remained standing during the trial. Heart rate rose gradually as the chamber temperature fell, peaking at a level 70 - 90 % higher than during thermoneutral conditions. Body core temperature was maintained or slightly increased during cold exposure (Figure III.6).

In contrast, Animal B spent a large portion of the experimental period in a lying position. While heart rate increased, peaking at a level 50 - 70 % higher than that recorded in a thermoneutral environment, changes in heart rate did not parallel the pattern of change in ambient temperature. As well, this animal allowed its body core

Figure III. 6 Effect of Acute Cold Exposure on Mammary Blood Flow, Heart Rate and Rectal Temperature of Animal A, Experiment I

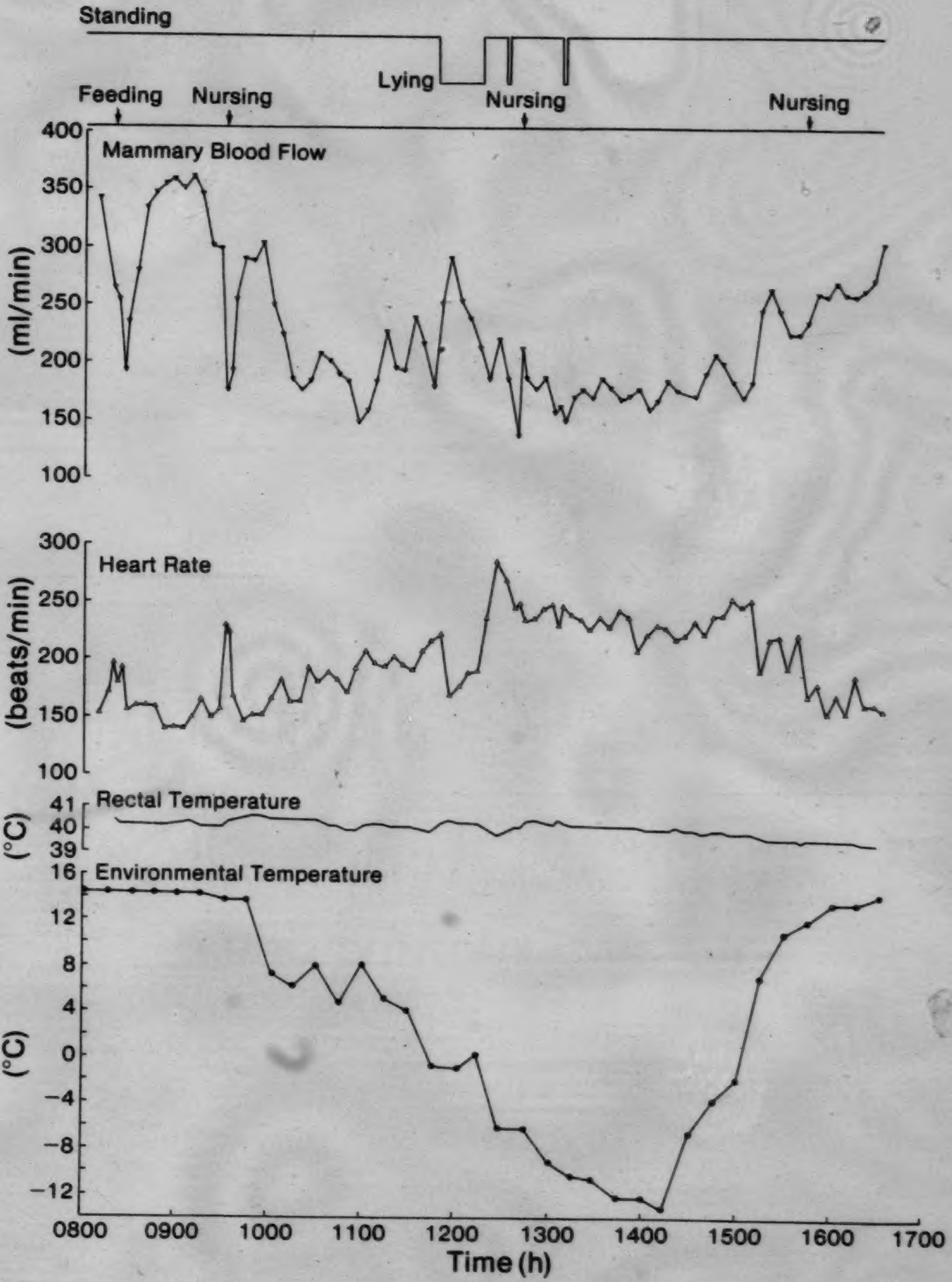


Figure III.7 Skin Surface Temperatures During a Period of Acute Cold Exposure, Animal A.

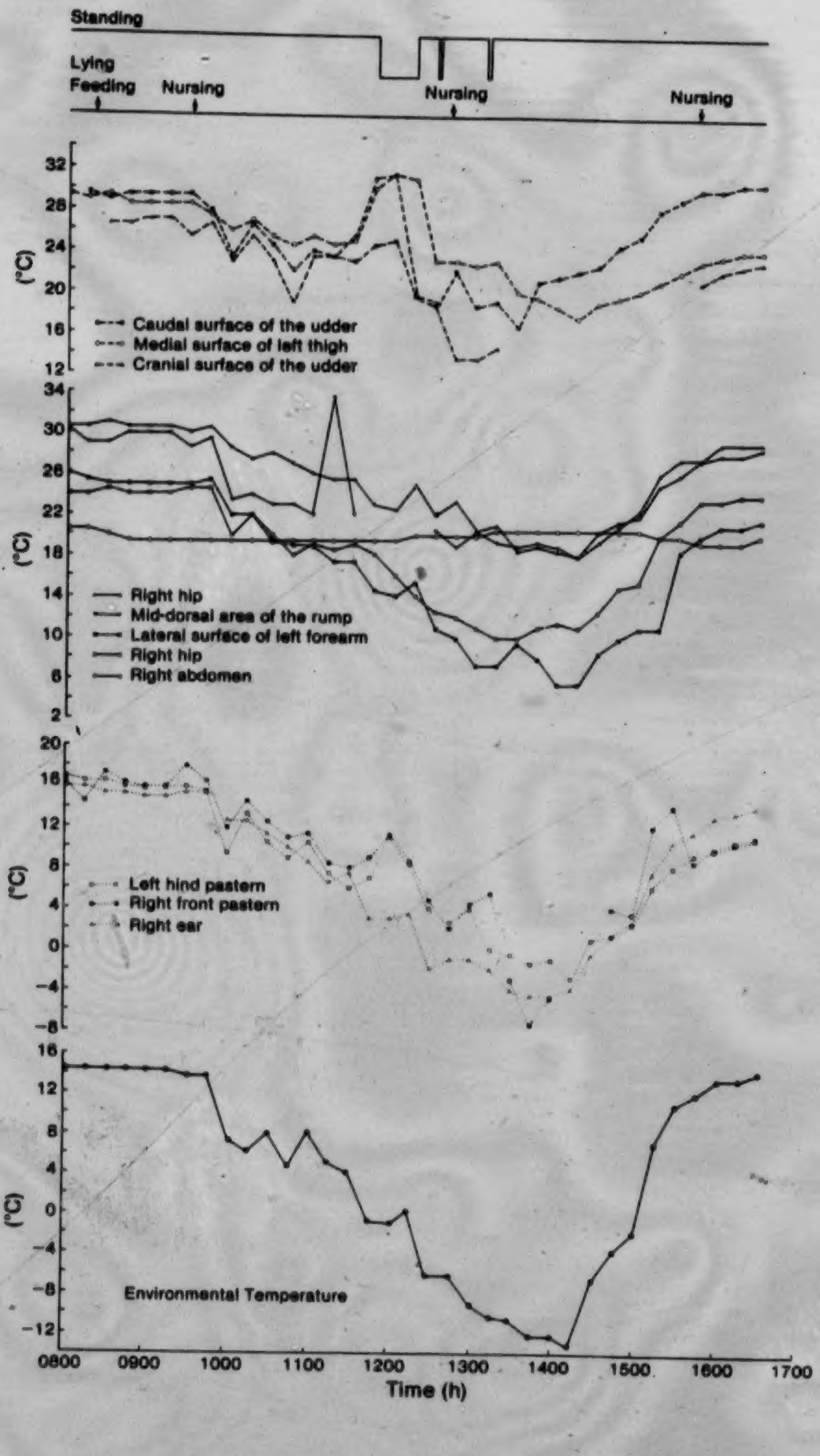


Figure III. 8 Effect of Acute Cold Exposure on Mammary Blood Flow, Heart Rate and Rectal Temperature of Animal B, Experiment I

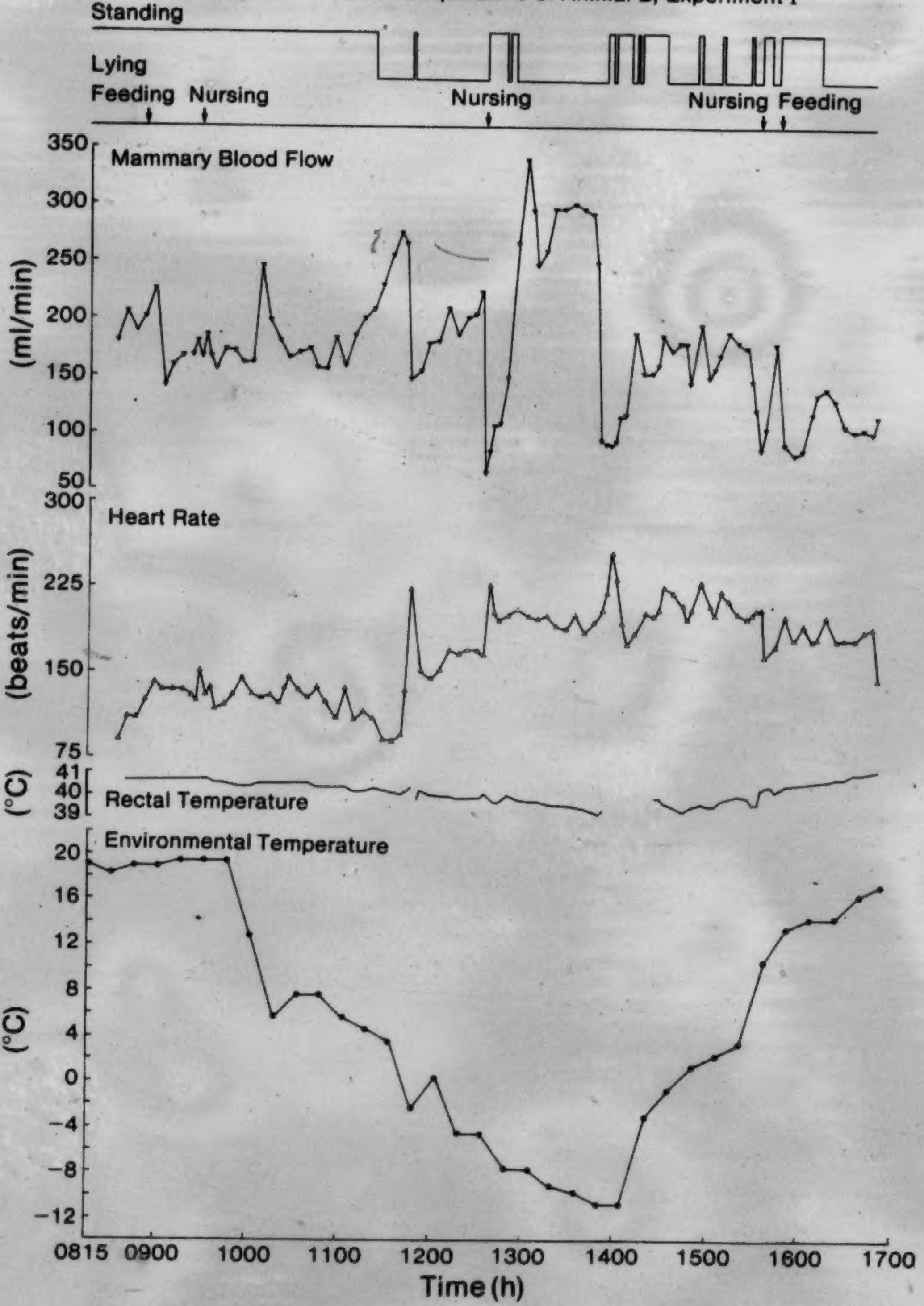
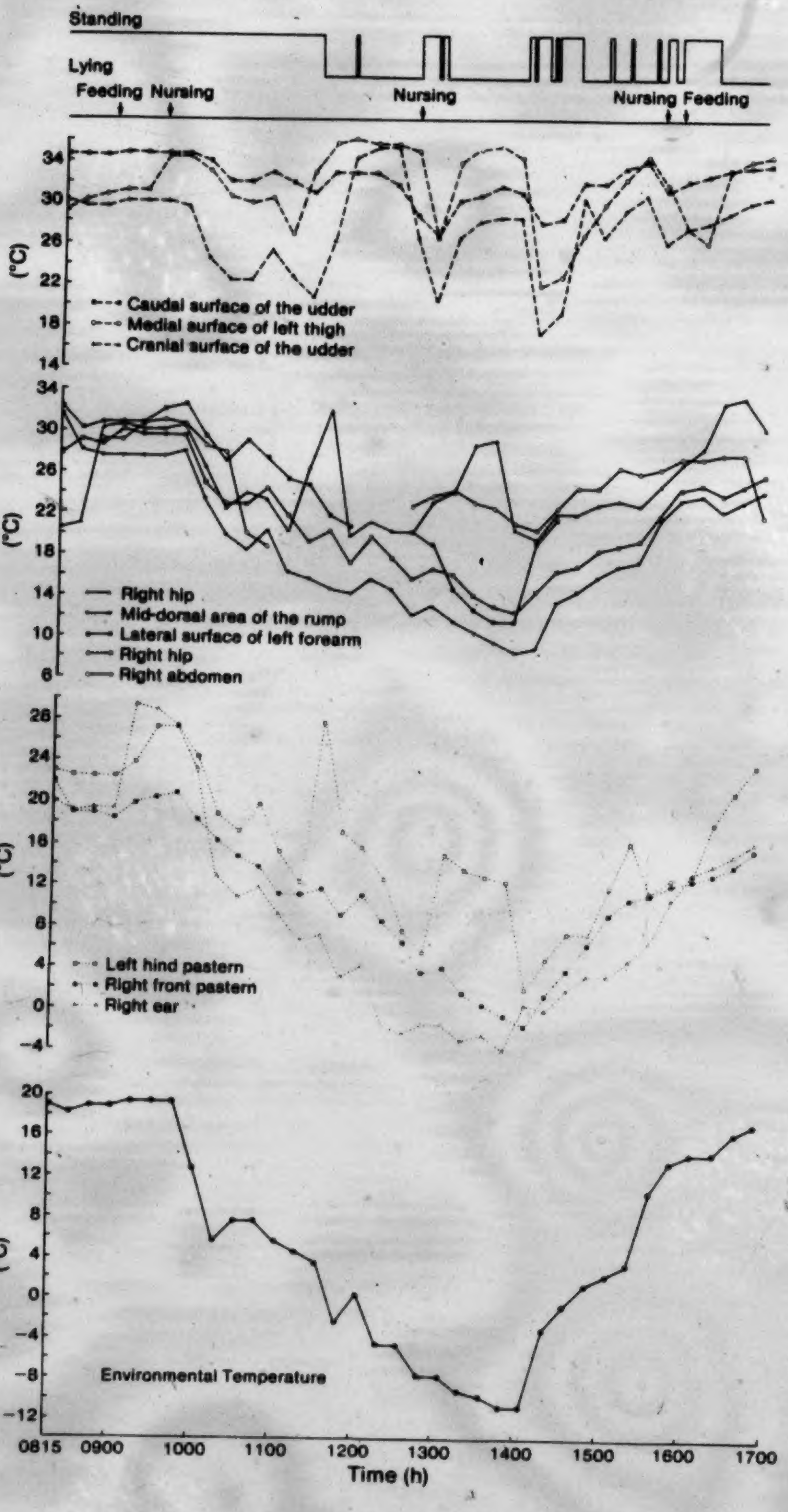


Figure III.9 Skin Surface Temperatures During a Period of Acute Cold Exposure, Animal B.



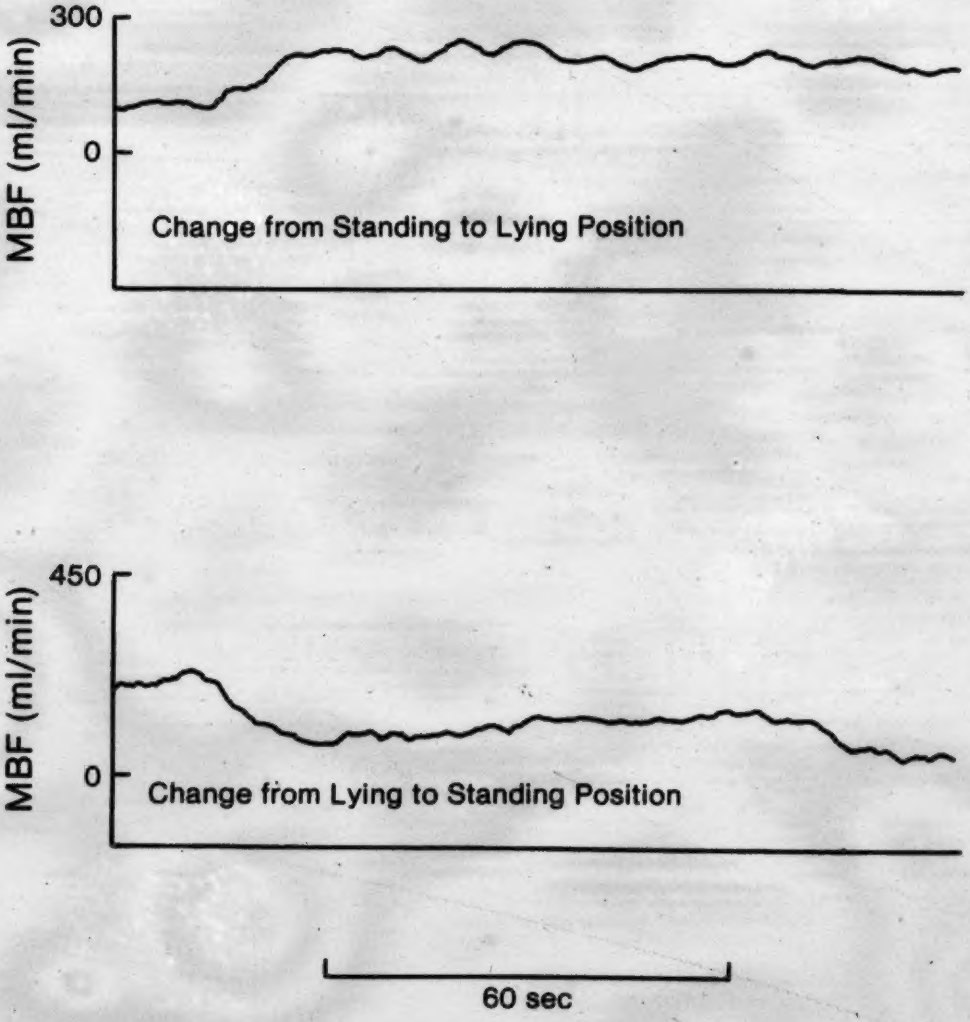
temperature to fall; rectal temperature decreased from a basal level of 40.6 °C to 38.8 °C, measured at the coldest chamber temperature (Figure III.8).

Skin temperatures measured during the period of cold exposure generally mimicked the trends in ambient temperature (Figures III.7 and III.9). Temperatures of the extremities decreased the most during the cold trial while temperatures recorded on the trunk surfaces fluctuated over a much narrower temperature range. Udder surface and thigh temperatures demonstrated the least degree of reduction during chamber cooling and were noticeably affected by postural changes. Feeding and suckling had little or no effect on any of the surface temperatures recorded.

As during thermoneutral conditions, MBF measured at a low ambient temperature was influenced by both posture and nursing activity. Lying down resulted in a rapid and sustained increase in MBF (Figures III.6, III.8, III.10). A change from lying to a standing position had an opposite effect on MBF. The influence of posture on MBF was more apparent for Animal B who changed positions frequently: the highest MBF values recorded occured at an ambient temperature of -11 °C when the ewe was lying down. Nursing again resulted in a rapid but transient decrease in MBF. This effect was more noticeable before the temperature in the chamber was reduced.

The influence of cold exposure on MBF is difficult to discern from the other confounding factors. Table III.2

Figure III.10 Effect of Posture on Mammary Blood Flow During a Period of Acute Cold Exposure, Experiment I.



presents heart rate and MBF data for the two animals measured before the chamber temperature was lowered and at selected points during the experimental period when ambient temperature, posture and time elapsed were similar for both animals. Although only two animals were used in these trials, the variation in MBF as influenced by ambient temperature is apparent. As the chamber temperature fell, MBF noticeably decreased in Animal A beginning at a threshold environmental temperature of approximately 7.5 °C. The same trend in reverse was apparent as the chamber temperature rose. Since Animal B spent most of the time in a lying position it is difficult to establish a standing MBF-temperature threshold estimate. From Table III.2 it is evident that as the chamber temperature fell, MBF was not affected at an ambient temperature of 7.0 °C, although it was markedly reduced at a chamber temperature of -8.0 °C. During re-warming of the chamber, MBF had recovered to pre-treatment values by an ambient temperature of -1.5 °C.

Experiment II

Prolonged cold stress resulted in a tendency towards a smaller mammary gland weight. This effect was primarily attributable to a reduction in secretory tissue weight, which approached statistical significance ($P<0.10$) (Table III.3).

Since the determination of MBF using the microsphere technique relies on the correct positioning of the injection and sampling catheters, their placement was verified during

Table III.2 The Effect of Low Ambient Temperature on the Heart Rate and Mammary Blood Flow of Lactating Ewes. Experiment I

Animal A

Time (h)	Posture[1]	Chamber Temperature °C	Heart Rate beats/min	Heart Rate % of control	MBF ml/min	MBF % of control
0815-0945	S	14.0[2]	107.5±10.2[3]	100	335.2±27.4[3]	100
1000	S	7.5	137	127	230	69
1300	S	- 8.5	200	186	162	48
1400	S	-12.0	182	169	173	52
1500	S	2.0	203	189	187	56

Animal B

Time (h)	Posture[1]	Chamber Temperature °C	Heart Rate beats/min	Heart Rate % of control	MBF ml/min	MBF % of control
0830-0945	S	18.5[2]	130.2±15.5[3]	100	180.2±20.8[3]	100
1030	S	7.0	131	101	178	99
1245	S	- 8.0	200	154	117	65
1400	S	-11.0	189	145	123	68
1500	S	- 1.5	227	174	176	98

[1] S=standing.
[2] control value.
[3] value represents mean±SEM.

Table III.3 Right Udder Half Tissue Weights of the Control and Cold-Exposed Ewes, Experiment II[1]

	CON (5)[2]	CE (4)
Bodyweight (kg)	34.2± 1.59	40.5± 3.78
Udder Half Weight		
(g)	242.7±22.50	235.1±49.50
(g/kg BW)	7.09± 0.49	5.64± 0.71
Secretory Tissue Weight		
(g)	163.4±18.97	144.9±28.66
(g/kg BW)	4.77± 0.43	3.48± 0.40†
Udder Skin Weight		
(g)	36.3± 3.30	44.1± 8.49
(g/kg BW)	1.06± 0.10	1.06± 0.12
Connective Tissue Weight		
(g)	35.9± 5.02	37.9±11.80
(g/kg BW)	1.05± 0.14	0.90± 0.21
Teat Weight		
(g)	3.9± 0.34	5.0± 0.62
(g/kg BW)	0.11± 0.01	0.12± 0.01
Lymph Node Weight		
(g)	3.1± 0.76	3.0± 0.85
(g/kg BW)	0.09± 0.02	0.07± 0.01

[1] values represent mean±SEM.

[2] number of animals/treatment in parentheses.

† treatment means are significantly different (P<0.10).

post-mortem examination. Zero or abnormally low counts in the mammary tissue were also used as an indication of improper catheter placement. For these reasons, the results from three animals (2 CON, 1 CE) were discarded. Furthermore, blockage of the injection catheter prevented the administration of microspheres to one animal in the CE group and of the second dose of microspheres (^{95}Nb) to one animal in the CON group.

Total blood flow to half the udder was reduced by approximately 32 % as a result of an 8 wk cold exposure (Table III.4). Part of this reduction reflects the slightly decreased size of the mammary gland of the CE animals however MBF expressed per unit of mammary weight also tended to be lower for the cold-exposed ewes. Treatment differences were not statistically significant, reflecting the high degree of individual variation in MBF.

Blood flow/unit weight of secretory tissue, lymph nodes and teat was not significantly different between treatments but on average tended to be lower in the CE ewes. A decrease in udder skin blood flow/100 g (10 min after suckling) and an increase in connective tissue blood flow/100 g (3 h after suckling) in the CE ewes approached statistical significance ($P<0.10$).

MBF measured 10 min after suckling was 19 - 20 % lower (statistically not significant) for both treatment groups than MBF measured 3 h after suckling (Table III.4). Again individual results varied widely. Except for the CON teat

Table III.4 Blood Flow to the Tissues of the Right Udder Half of the Control and Cold-Exposed Ewes, Experiment II[1]

	10 Min After Suckling (^{95}Nb) CON (2)[2]	10 Min After Suckling (^{95}Nb) CE (3)	3 h After Suckling (^{141}Ce) CON (3)	3 h After Suckling (^{141}Ce) CE (3)
Blood Flow (ml.min^{-1})				
Udder Half	123.6±29.63	84.2±33.00	154.2±75.95	104.2±47.76
Secretory Tissue	111.1±13.79	70.7±20.03	142.5±53.29	88.1±27.78
Lymph Nodes	6.3± 4.25	5.5± 2.17	6.2± 1.13	6.0± 2.83
Skin	2.7± 0.18	1.9± 0.37	3.6± 1.67	2.1± 0.34
Teat	1.1± 0.71	0.3± 0.08	0.4± 0.21	0.4± 0.17
Connective Tissue	2.4± 2.37	5.6± 2.21	1.5± 0.88	7.5± 3.29
Blood Flow (ml.100g^{-1}.min^{-1})				
Udder Half	52.7±12.87	37.4±15.82	54.3±15.75	44.3±16.97
Secretory Tissue	73.2±11.75	55.0±36.39	75.6±35.05	65.3±38.40
Lymph Nodes	169.2±58.38	150.3±16.78	176.6±36.12	163.8±49.46
Skin	8.5± 1.37*a*	4.3± 0.68*b*	9.6± 3.65*ab*	4.8± 0.84*b*
Teat	24.9±13.03	5.9± 0.96	9.9± 5.62	8.0± 1.90
Connective Tissue	6.1± 6.05*ab*	14.4± 6.06*ab*	3.6± 2.25*b*	17.6± 4.94*a*

[1] values represent mean±SEM.

[2] number of animals/treatment in parentheses.

a,b means within a row followed by a different letter are significantly different ($P<0.10$).

and connective tissue blood flows, in both treatment groups a tendency of reduced MBF as a result of suckling was mirrored in all of the mammary tissues.

The distribution of MBF within the udder tended to be altered as a result of cold exposure (Table III.5). As a percentage of total MBF, secretory tissue blood flow was lower but connective tissue and lymph node blood flows higher for the cold-exposed ewes. Skin and teat blood flows were not affected to any extent by thermal treatment. In both treatment groups, secretory and lymphoid tissues received a greater percentage of the total mammary blood flow than could have been predicted from their proportion of the total mammary weight.

Since microspheres would have been distributed to every area supplied with blood from the right external iliac artery, blood flow to selected tissues of the right hindlimb was examined (Table III.6). Time of injection relative to suckling had no influence on blood flow to these regions; consequently, results from both microsphere injections are included together. Cold exposure resulted in a decreased blood flow to the skin of the thigh ($P<0.05$) and of the lower leg ($P<0.10$). Blood flow to two selected muscles, the gastrocnemius and semimembranosus, was increased in the CE group although treatment differences were not significant ($P>0.05$). A significant treatment difference ($P<0.01$) was apparent in the blood flow to the popliteal fat depot of the hindlimb; cold exposure resulted in over a 300 % increase of

Table III.5 Distribution of Weight and Blood Flow Among the Mammary Tissues of the Control and Cold-Exposed Lactating Ewes, Experiment II'

Mammary Tissue	% Mammary Weight		% Mammary Blood Flow	
	CON	CE	CON	CE
Secretory Tissue	67.3	61.6	91.2	84.3
Skin	15.0	18.8	2.3	2.2
Connective Tissue	14.8	16.1	1.6	7.0
Teat	1.6	2.1	0.6	0.4
Lymphoid Tissue	1.3	1.3	4.6	6.2

' values represent the mean of the results obtained from 5 CON and 4 CE animals.

Table III.6 Blood Flow to Selected Tissues of the Right Hindleg of the Control and Cold-Exposed Lactating Ewes, Experiment II[1]

Tissue Blood Flow ($ml.100g^{-1}.min^{-1}$)	CON (5)[2]	CE (6)
Thigh Skin	17.99±2.28	10.54±1.72*
Lower Leg Skin	2.06±1.10	0.18±0.08†
Gastrocnemius	12.92±4.07	22.24±5.77
Semimembranosus	17.38±7.88	22.45±9.50
Popliteal Fat Depot	8.58±3.47	31.65±4.44**

[1] values represent mean±SEM.

[2] number of observations/mean in parentheses.

† treatment means are significantly different ($P<0.10$).

* treatment means are significantly different ($P<0.05$).

**treatment means are significantly different ($P<0.01$).

blood flow to this area.

Samples of tissue from the left half of the udder contained an insignificant amount of radioactivity, indicating that blood flow between udder halves was minimal. Microspheres were found in lung tissue samples as a result of passage through arterio-venosus anastomoses located either in the udder or in the right hindlimb. Negligible amounts of radioactivity were found in the kidney samples, suggesting that few microspheres passed through the lungs to be recycled into the systemic circulation.

E. Discussion

Values for total blood flow to the udder of the ewe housed in a thermoneutral environment have been previously obtained during pregnancy, using electromagnetic blood flow probes (Burd et al. 1975;1978a,b), and during lactation, by using the microsphere technique (Thompson 1980) and by applying the Fick principle to measurements of mammary uptake of methionine from the bloodstream (Davis and Bickerstaffe 1978). Unilateral MBF averaged 300 $ml.min^{-1}$ just prior to parturition (Burd et al 1975) and 358 $ml.min^{-1}$ at mid-lactation (Thompson 1980). Total MBF measured by Davis and Bickerstaffe (1978) averaged 930 $ml.min^{-1}$ in lactating ewes.

Generally, thermoneutral MBF values obtained during the present studies were lower than those values previously reported (Experiment I unilateral MBF range= 80 - 335 $ml.min^{-1}$; Experiment II unilateral MBF range= 50 - 230 $ml.min^{-1}$ $\bar{X}$= 142 $ml.min^{-1}$). Large variations in blood flow and udder size were encountered in both the current and in other studies. As well, discrepancies between reported MBF values may arise from such factors as stage of lactation, number of young being nursed, breed and age of the ewes.

MBF values expressed per 100 g wet mammary weight removes some of the sources of variation in blood flow results. In lactating ewes housed in thermoneutral conditions, published values of 67 $ml.100\ g^{-1}.min^{-1}$ (27 - 36 days post-partum) (Thompson 1980) and 107 $ml.100\ g^{-1}.min^{-1}$

(8 - 71 days post-partum) (Davis and Bickerstaffe 1978) are comparable with an average MBF of 50 ml.100 g^{-1}.min^{-1} (55-63 days post-partum) found for the CON ewes in Experiment II. The lower values of MBF/100 g mammary tissue found in the present study might be expected since the ewes were nearing the end of a normal lactation period.

The tissue composition of the mammary gland for both treatment groups in Experiment II was quite dissimilar to that published previously for lactating ewes. Thompson (1980) reported that secretory tissue formed 87 % and 61 % of the total mammary weight in the lactating and non-lactating ewe, respectively. In the present study mammary tissue composition more closely resembled that of the non-lactating ewe. This discrepancy may be due to the stage of lactation: the animals examined by Thompson (1980) were at peak lactation whereas the ewes in the present study were in declining lactation at the time of measurement.

Thompson (1980) determined that, of the total MBF in lactating ewes housed at a neutral temperature, secretory tissue received 98.2 %; adipose tissue, 0.64 %; lymph nodes, 0.65 %; teat, 0.10 %; and udder skin, 0.41 %. A similar MBF distribution was found for the CON ewes in Experiment II, although secretory tissue flow accounted for only 91.2 % of the total MBF. Again, differences in MBF distribution may be related to the stage of lactation. Other discrepancies might also have resulted from a difference in dissection technique: Thompson (1980) found a distinct inguinal fat depot in the udder of the lactating ewe whereas in the present study, no distinct depot was located, although adipose tissue was found diffusely spread throughout the connective tissue.

The diurnal variation in MBF for lactating sheep (Experiment I) was greater than that found in other studies with lactating goats (Linzell 1974; Burvenich 1979). Possibly the large variation in the current study was due to some of the experimental conditions imposed.

The influence of posture on MBF has not been previously examined since, in many studies, the animals are prevented from lying down. The increased MBF when the ewe laid down could have resulted from a restriction of blood flow to the other branches of the external iliac artery or from a change in general activity and hormonal status.

The present data indicates that MBF is sensitive to even minor disturbances of the animal. Burd et al. (1975) noted that MBF measured in sheep fell when the experimenter entered the room. Other studies have confirmed that MBF in all animals drops in response to mild stress (Linzell 1974). More detailed studies have demonstrated that low doses of adrenaline (4 - 10 ng/kg BW) and noradrenaline (16.6 - 35.7 ng/kg BW) cause marked vasoconstriction in the mammary artery of lactating goats and ewes (Houvanghel 1970). The same response has been observed in dairy cows (Dhondt et al. 1973) and in mammary-lung perfusions (Hebb and Linzell 1951).

In Experiment I, a decrease in blood flow to the mammary gland was noted when the experimenter approached the animal however a further decrease in MBF occured as a result of nursing. During Experiment II, MBF tended to be decreased 10 min after suckling in 4 of the 5 animals in comparison to MBF measured 3 h after suckling, even though the experimenter was in close proximity to the animals for both measurements.

Conflicting results exist in the literature concerning the effects of milking or suckling on MBF. Burd et al. (1975) reported that when ewes were milked, blood flow through the mammary artery decreased. Since this study was performed with pregnant ewes, presumably milk was withdrawn from the mammary gland either by machine or hand-milking. With either technique, a great deal of stress could have been imposed if the ewes were not accustomed to the procedure. Burvenich (1979) determined that MBF showed a parabola-shaped daily variation in lactating dairy goats milked twice daily, the lowest values being recorded immediately after milking. Hanwell and Linzell (1973b) also found that the lowest MBF values measured in anesthetized rats 15 days post-partum, following a 24 h separation from their young, corresponded to the lowest levels of milk accumulation. In contrast, Houvenaghel et al. (1973) reported that handmilking and udder massage in dairy cows resulted in higher MBF values. Blood flow to the mammary gland also has been found to increase in rabbits in response to suckling (Katz and Creasy 1982).

Since suckling results in the release of prolactin (suckling reflex) and oxytocin (milk ejection reflex) (see reviews by Grosvenor and Mena 1974; Ensor 1978), it is possible that these hormones may influence MBF. When prolactin release was inhibited by the administration of ergocryptine in pregnant ewes, normal increases in MBF occured when parturition was induced (Burd et al. 1978). These findings suggest that prolactin was not an important factor in the MBF changes observed in the ewe at the time of parturition. Hanwell and Linzell (1973b), using an indicator fractionation method in anaesthetized rats, determined that MBF decreased when the young were removed from their dam's cage. This decrease in MBF was attributed to the withdrawal of the suckling stimulus since, when the pups were allowed to remain in the cage and suckle sealed teats, MBF did not fall. From these results Hanwell and Linzell (1973b) postulated that prolactin release was necessary to maintain a high rate of blood flow through the mammary gland in lactating rats. This conclusion perhaps was unfounded since the results observed when the pups were removed may have been caused by maternal stress and adrenaline release rather than by the removal of the suckling stimulus.

When oxytocin is administered to cows in either physiological or pharmacological doses, blood flow to both empty and engorged udders has been found to increase (Houvenaghel et al. 1973). The influence of oxytocin on MBF in goats and sheep however has been reported to be variable (Dhondt et al. 1973). The possible contributing influence of endogenous prolactin and oxytocin release on the decreased MBF observed in ewes as a result of suckling in the present experiments therefore cannot be properly assessed at this time.

Although total mammary blood flow in the CON lactating ewes in Experiment II tended to fall as a result of suckling, changes in mammary tissue blood flows were variable. Suckling resulted in a tendency of decreased blood flow to the secretory and skin tissues and increased blood flow to the teat. Alterations in lymphoid and connective tissue blood flows were not apparent. Since measurements from only two CON animals were available, it is not known how representative these responses are. The observed tendency of a decreased total mammary blood flow but an increased flow to the teat after suckling would support the hypothesis of Nisbet (1956) that blood flow to the teat can be locally controlled.

During continuous MBF recordings in the ewe (Experiment I), blood flow remained relatively stable between periods of suckling, showing no consistent tendency to increase or decrease as the udder became engorged with milk. The influence of mammary engorgement on MBF has been studied in anaesthetized rats (Hanwell and Linzell 1973b) and mice (Mao and Caruolo 1973). Both studies determined that when the young were separated from the dam for an extended period (12 or 24 h), MBF was negatively correlated with milk accumulation in the mammary gland. The effect of mammary engorgement on MBF in dairy goats has been examined by infusing air (Pearl et al. 1973) or isosmotic sucrose solution (Peaker 1980) and by allowing the gland to fill with milk at the end of lactation (Fleet and Peaker 1978). While all of these studies revealed that high levels of intramammary pressure could result in a reduced blood flow to the mammary gland, it is doubtful that sufficient pressure would develop in a lactating goat milked twice daily to result in any change in MBF. Possibly then a 3 h accumulation of milk in the udder of the ewe also causes an insufficient increase of pressure to adversely affect MBF.

Changes in MBF relative to feeding have not been previously examined. The reduced blood flow seen in the present study at the time of feeding could be due to the release of adrenaline as a result of the presence of experimental personnel. As well, recent evidence suggests that there is an endogenous release of noradrenaline and adrenaline in association with feeding in cattle (Christopherson et al. 1982) which may also induce a reduction in blood flow through the mammary artery. Increased MBF approximately 4 h after feeding may reflect a higher availability of milk substrates at this time as a result of digestive and absorptive processes.

Decreased MBF in the lactating ewe during periods of acute cold stress agrees with other work performed with lactating goats. Thompson and Thomson (1977), Thomson et al. (1979) and Faulkner et al. (1980) have used a dye dilution technique to measure MBF in acutely cold-stressed dairy goats. In all three studies, mild cold stress had no influence on MBF but a 24 or 48 h exposure to a moderate cold stress, sufficient to increase resting metabolic rate by 46 - 50 %, resulted in a 24 - 46 % decrease in MBF.

Although the metabolic rates of the ewes acutely exposed to a low ambient temperature were not measured in Experiment I, the level of cold stress imposed was sufficient to cause marked vasoconstriction, as indicated by the recordings of surface temperatures, and resulted in visible shivering of the animals. Previous data presented in Chapter II has shown that the metabolic rates of lactating ewes housed at 0 °C were increased by 20 - 55 %, depending upon the duration of cold exposure, suggesting that the severity of cold exposure inflicted in Experiment I also could be classified as moderate.

MBF measured during cold stress was also affected by minor disturbances, suckling and postural changes. Both mild stress and suckling resulted in a decreased flow through the mammary artery however the effects were not as obvious during a period of cold exposure as they were at control temperatures. It is possible that a change in MBF due to mild stress or suckling in these trials may have been partially masked by a cold-induced constriction of the mammary artery.

Indirect evidence of a depressed blood flow to the udder during cold exposure was obtained from the recordings of mammary temperature. Surface temperature measured from the cranial and caudal portions of the udder fell respectively, by 12.5 °C and 12.0 °C in Animal A and 13.5 °C and 6.0 °C in Animal B during the period of cold exposure. This decrease in udder surface temperature is larger than that reported previously for acutely cold-stressed sheep (Smith et al. 1978) however, a more severe cold stress may have been imposed in the present study.

Lying down effectively insulated the mammary gland from a low ambient temperature as evidenced by the increase in MBF and udder surface temperatures. Smith et al. (1978) also noted similar trends in the udder surface temperatures of cold-stressed lactating ewes. They concluded that lying down resulted in an increase in udder surface temperature by either affecting MBF or by decreasing surface conductive and convective heat losses. The present study confirms that posture can affect MBF and probably mammary heat loss, although the influence of posture on the rate of heat loss from the mammary gland remains to be quantitatively examined.

Prolonged cold exposure (Experiment II) tended to result in a reduction of total mammary weight, especially when expressed as a percentage of bodyweight (Chapter II). The possible contribution of a smaller udder size to the thermal balance ot the animal is uncertain. Smith et al.

(1978) suggested that despite the relative lack of insulation, the mammary gland of the ewe can only account for a small portion of the total heat loss of the cold-exposed animal. Further work is necessary to support this conclusion.

After accounting for mammary weight differences, total MBF was lowered by 18 - 29 % as a result of prolonged cold exposure. Although this result was not significant, it is slightly lower than the decrease observed previously in acutely cold stressed lactating goats (Thompson and Thomson 1977; Thomson et al. 1979; Faulkner et al. 1980). In the CE animals, suckling tended to further reduce total MBF, a trend that was mirrored in all mammary structures.

The influence of cold exposure on mammary blood flow distribution has only been examined previously in the non-lactating animal. Thompson (1980) determined that reductions in udder skin and teat blood flows in non-lactating ewes exposed to 0 °C for 2 h was approximately balanced by an increased blood flow to the mammary adipose tissue. In the present experiment, a cold-induced reduction in blood flow to all udder tissues except for the lymphoid and connective tissues was observed. An increased proportion of blood flow to the connective tissue of the udder perhaps supports Thompson's (1980) observation of a greater blood flow to the mammary fat depot since in the current experiment, adipose tissue was included in the classification of connective tissue.

Of the tissues of the hindlimb examined in the present study, a very large increase in blood supplied by the external iliac artery to the popliteal fat depot was observed as a result of cold stress. A cold-induced elevation in blood flow to adipose depots in mature sheep has also been found by Hales et al. (1976) for the perirenal fat depot and for the hindlimb fat in cattle (Bell et al. 1976), possibly supporting an increased mobilization of white adipose tissue as discussed by Thompson (1977).

Blood flow to the skin of the hindlimb of the chronically cold stressed ewes tended to be lower whereas blood flow to the hindlimb muscles tended to be elevated in comparison to the values obtained in the CON animals. Microsphere studies of blood flow to the hindleg of the young ox after acute cold stress have revealed a non-significant decrease in skin blood flow but an increased flow to the muscles (Bell et al. 1976). Hales et al. (1976) discovered that leg skin blood flow fell to an extremely low level in adult sheep exposed for approximately 60 min to a cold environment although blood flow to the muscles of the upper hindlimb increased. In contrast, Schaefer et al. (1982) failed to demonstrate any reduction in leg skin blood flow in adult sheep as a result of acute or chronic cold stress although blood flow to the gluteus maximus muscle of the hindleg was elevated after prolonged cold exposure. Discrepancies between previously reported results may stem from differences in environmental and experimental conditions. The thermal balance of a cold-stressed animal would tend to favor a reduction in blood flow to the skin of the extremities to minimize heat loss to the environment but an elevated flow to the skeletal muscles to support shivering thermogenesis (Thompson 1977).

The present studies have indicated that at least part of the diurnal variation in MBF can be related to feeding, posture and suckling although the underlying control mechanisms involved are uncertain. As well, blood flow to the mammary gland was found to be sensitive to cold and other forms of stress. Further research is necessary to determine if the duration of cold exposure has a significant influence on the level of inhibition of blood flow through the mammary artery. The importance of a reduction in MBF during prolonged cold exposure in relation to the maintenance of thermal equilibrium and to the process of milk formation in the ewe also will require additional examination.

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IV. Section III

The Effects of Prolonged Cold Exposure on the Growth, Digestion and Morphological Development of Young Lambs

A. Abstract

The performance and development of young lambs housed at 0±1 °C or 21 ±1°C were compared in two experiments. Despite similar nutrient intakes for both treatment groups, growth rate of the cold-exposed lambs was depressed by 9-16 % prior to weaning and by 20 % shortly after weaning. By 10 weeks of age morphological differences between the two groups were apparent, especially in terms of smaller ear dimensions ($P<0.01$), head ($P<0.10$) and metatarsal lengths ($P<0.05$) of the cold-exposed lambs. Thirteen week old weaned lambs also tended to have shorter leg bones than those housed at 0 °C. Dry matter and nitrogen digestibilities of a concentrate ration fed to the weaned lambs were not affected by treatment although nitrogen retention (g/day,g/gDNI) was significantly lowered ($P<0.05$) as a result of cold exposure. Organ weights measured after 11 wk of differential temperature exposure were similar for both groups except for slightly increased abomasal and thyroid weights ($P<0.10$) in the cold-exposed group.

B. Introduction

The exposure of a homeotherm to a cold environment results in marked physiological responses (see reviews by Thompson 1977; Alexander 1979) that enable it to adapt to the unfavorable conditions. Due to a change in the rate of heat exchange between the animal and the environment, the cold-exposed animal must strive to minimize heat loss and augment heat production in order to maintain a state of thermal equilibrium (Mitchell 1974; Webster 1974). Under these conditions, the maintenance requirement of the animal has been shown to increase (Graham et al. 1959; Young and Christopherson 1974) and, as a consequence, animal productivity may suffer.

After accounting for the direct effect of cold exposure on the metabolic requirements of the animal, the depression of performance that occurs in a cold environment appears largely to be nutritionally related. Cold-stressed animals will increase their voluntary food intake (Baile and Forbes 1974) but digestibility of the feed often is reduced (NRC 1981; Kennedy et al. 1982); as a result, the net supply of nutrients available to meet the metabolic demands of the animal may be only slightly increased when food is available ad-libitum, or may even be reduced if food intake is restricted. Studies of the digestive responses of ruminants to cold stress performed to date however have concentrated mainly on animals at maintenance and little work has been done on productive (ie. growing, pregnant, lactating) animals.

While a young growing ruminant may potentially be more susceptible to stress and insufficient nutrient availability than a mature animal, a growing animal might also be better able to adapt to a thermal stress, thereby reducing the degree of environmental discomfort experienced. For example, a reduction in the growth of the extremity areas may help to minimize the heat loss of the animal. Although, generally, morphological adaptations to cold are considered to be genetically controlled, Weaver and Ingram (1969) and Heath and Ingram (1979) have reported that a relatively short term exposure of growing pigs to a cold environment can result in marked alterations in development.

The present experiments were conducted to determine the response and adaptative capacity of young lambs to a cold environment by examining growth, body dimensions, organ weights, and nitrogen digestibility and retention during the first 13 weeks of life.

C. Materials and Methods

Experiment I

Four crossbred Suffolk lambs (2 male, 2 female) were housed with their dams from wk 1-9 of age at each of two exposure temperatures. Control (CON) lambs were housed in an animal room at a temperature of 21±1 °C while the cold-exposed (CE) lambs were housed within one of two temperature-controlled chambers maintained at 0±1 °C. Each lamb and ewe pair was kept in a metabolic crate (1.2x1.2 m) designed to deny access of the lamb to the ewe's food. Growth of the lambs was monitored by weekly weighings. Morphological development was assessed twice during the experimental period when the lambs averaged 4 and 9 wk of age. Leg bone lengths were determined in duplicate by flexing the joints of the leg and measuring the inter-joint distance with calipers. Other measurements (head length, body length) were determined using a tape measure.

Experiment II

A second experiment was performed to further examine the growth rate and to determine the digestive responses of lambs housed in a cold environment. Five crossbred Suffolk lambs (3 male, 2 female) were assigned to each of two treatment groups: treatment 1 lambs (CON) were housed at an ambient temperature of 21±1 °C within two continuously lit rooms, while treatment 2 lambs (CE) were kept in two temperature-controlled chambers maintained at a temperature of 0±1 °C. The experimental treatments began when the lambs averaged 12 days of age and continued for 11 weeks.

During the first eight weeks of the experiment each lamb was individually penned with its dam in a 1.0x1.8 m pen bedded with wood shavings. Throughout this period the only source of nutrition for the lamb was that supplied by the ewe's milk. Growth of the lambs was monitored by weekly weighings.

At 10 wk of age the lambs were weaned and duplicate measurements of various dimensions (wither height, head and ear length, ear width, leg and body length) were made with a tape measure on the live animals, using external anatomical structures and bony protuberences as landmarks.

Following weaning, each lamb was placed in a metabolic crate (0.61x1.52 m) and fed a concentrate ration (Table IV.1) at a level of 28 g .kg BW^{-1}.day^{-1} . This level of feeding was calculated to meet the nutrient requirements of a growing lamb housed at a thermoneutral temperature (NRC 1975). Unconsumed feed was collected and weighed and fresh feed offered twice daily (0800 & 1630 h). Water was available ad-libitum.

After an initial 10 day adjustment period, a digestibility-nitrogen balance trial was conducted. Over a 9 day period, total fecal and urinary output was collected. Fecal matter was retained by a screen located below the floor of the crate and sloped plastic sheeting used to direct the urine into a bucket containing an acetic acid-mercuric chloride solution (to prevent ammonia release

Table IV.1 Composition of the Concentrate Diet Fed to the Weaned Lambs, Experiment II

Ingredient:	% as fed
Rolled Barley	83.2
Soybean Meal	14.7
Cobalt-Iodized Salt	1.0
Calcium Phosphate	1.0
Vitamin ADE Premix	0.1

and bacterial action). Representative feed, fecal and urine samples were stored at -10 °C prior to analysis. Samples of feed and feces were dried to a constant weight in a forced air oven at 65 °C to determine dry matter content. Nitrogen anlaysis of the feed, fecal and urine samples was performed using the macro-Kjeldahl method (AOAC 1980).

At 13 wk of age the lambs were slaughtered and the carcasses dissected to determine organ weights and bone lengths. Bone lengths were determined in duplicate by using calipers to measure the distance between joint surfaces.

Statistical Analysis

Results from each experiment were analyzed separately. Treatment differences for weight gain, ADG, lamb dimensions, digestibility-nitrogen balance results and organ weights were compared using the Students t-test. Bonelengths of the 13 wk old lambs (Experiment II) were analyzed using bodyweight as a covariate when Least Squares Analysis of Variance showed that the covariate was significant (Steel and Torrie 1980).

D. Results

Experiment I

One lamb from the CE group was excluded from the analyses since milk intake from wk 5-9 of age was restricted by severe mastitis in the ewe. Weight gain over 49 days prior to weaning averaged 11.83±1.29 and 9.99±0.83 kg for the CON and CE groups respectively, representing a decrease of 16 % ($P>0.10$) as a result of cold exposure (Figure IV.1). Estimated daily intake of milk protein and energy respectively, averaged 78.7±2.27 g and 1.89±0.102 Mcal for the CON lambs, and 75.2±2.49 g and 2.04±0.071 Mcal for the CE animals during this period (Chapter II).

Bone development of the lambs was assessed by measuring the percentage change in bone length from 4 to 9 wk of age (Table IV.2). Although the percentage change in length for all of the parameters measured was lower as a result of cold exposure, only a treatment difference for metatarsal growth was statistically significant ($P<0.05$). By 9 wk of age ear area of the CE lambs visually appeared smaller but proved difficult to accurately measure (Plate IV.1). Differences between treatment groups were also observed in the external appearance of the nasal area of the head.

Experiment II

Weight gain over 57 days prior to weaning was reduced by 9 % ($P>0.10$) as a result of cold exposure (Table IV.3, Figure IV.2) although estimated daily nutrient intake during this period was similar for both treatment groups; from the

Figure IV.1 Growth of the Control (●——●) and Cold-Exposed (o– – –o) Lambs, Experiment I.

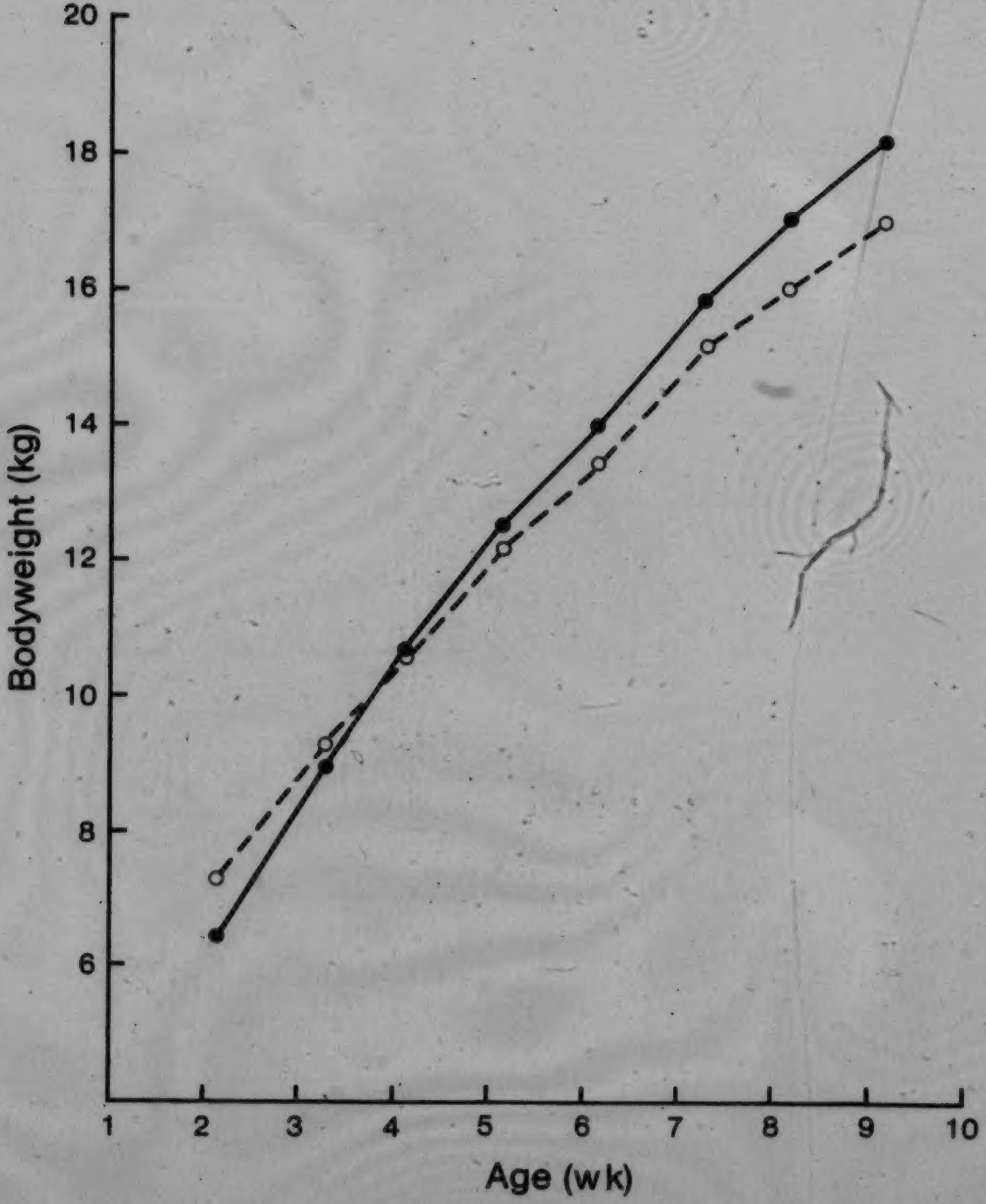


Table IV.2 Bone Growth[1] in Lambs From Week 4-9 of Age, Experiment I[2]

Measurement	CON (4)[3]	CE (3)
Weight Change (kg)	7.51±1.07	6.69±0.40
Right Radius	15.45±2.02	10.47±1.77
Right Metatarsus	8.30±0.85	5.30±0.50*
Right Tibia	29.45±8.05	20.47±4.48
Head	19.53±1.21	17.07±0.71
Body Length[4]	23.85±4.56	22.70±4.11

[1] bone growth measured as the percentage change in length over a 36 day period.

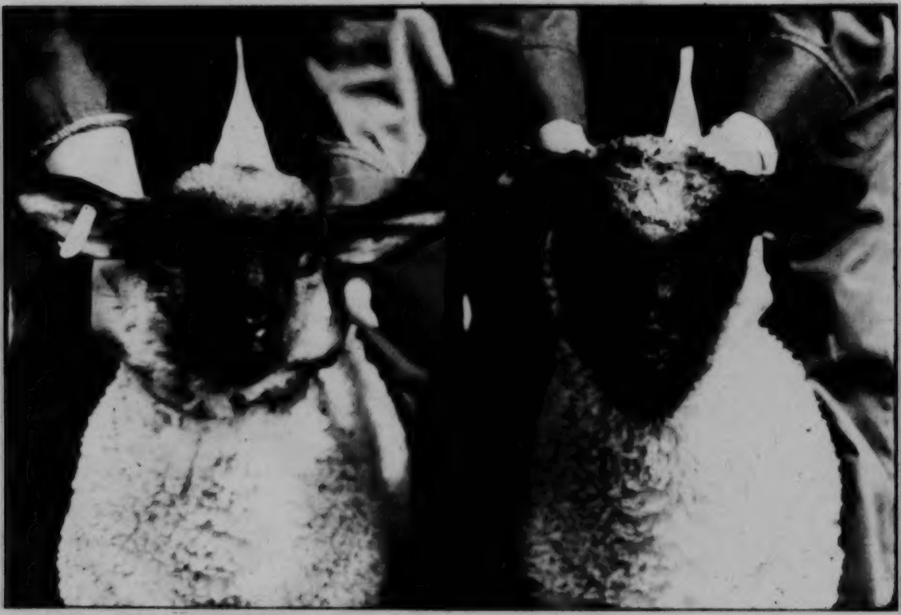
[2] values represent mean±SEM.

[3] number of animals/treatment in parentheses.

[4] body length measured from the point of shoulder to the point of buttock.

* treatment means are significantly different ($P<0.05$).

Plate IV.1 Appearance of the Ears and Nasal Area of a Control and a Cold-Exposed Lamb



CONTROL

COLD-EXPOSED

analysis of milk yield and composition, protein intake averaged 32.05±1.65 g/day and 33.26±2.67 g/day for the CON and CE lambs respectively, while energy intake for the CON and CE groups, respectively, averaged 0.80±0.055 Mcal/day and 0.87±0.073 Mcal/day. Pre-weaning growth rate was approximately 25 % lower during Experiment II than in Experiment I but can be related to a large difference in the average milk production of the ewes in the two studies (Chapter II). Weight gain measured over 11 days following weaning also was lower, but not significantly so ($P>0.10$) for the CE lambs. ADG was adversely affected by weaning for both treatment groups (Table IV.3).

Anatomical measurements performed on the live animals at 10 wk of age were consistently smaller for the CE lambs however only ear dimensions ($P<0.01$), hindleg ($P<0.01$) and head lengths ($P<0.10$) were significantly influenced by treatment (Table IV.4). Since the CE lambs on average weighed less than the CON lambs when these measurements were taken, treatment differences may reflect a smaller bodyweight rather than altered development.

Analysis of the bone lengths of the 13 wk old lambs, adjusted where appropriate for bodyweight, again revealed that cold exposure tended to result in a reduction in leg bone length, although the differences between treatments were not statistically significant ($P>0.10$) (Table IV.5).

Although DM and N intakes per kg bodyweight were equivalent for both treatment groups, the total DM and N

Table IV.3 Pre- and Post-Weaning Weight Gain of the Control and Cold-Exposed Lambs, Experiment II[1]

Measurement	CON (5)[2]	CE (5)
Pre-Weaning Weight Gain[3] (kg)	8.58± 0.89	7.81± 1.01
Post-Weaning Weight Gain[4] (kg)	1.30± 0.26	0.70± 0.37
Pre-Weaning ADG ($g.day^{-1}$)	150.5±15.57	137.1±17.73
Post-Weaning ADG ($g.day^{-1}$)	118.2±34.02	63.6±23.78

[1] values represent mean±SEM.

[2] number of animals/treatment in parentheses.

[3] values represent weight gain over 57 days.

[4] values represent weight gain over 11 days.

Figure IV.2 Growth of the Control (●——●) and Cold-Exposed (○– – –○) Lambs, Experiment II.

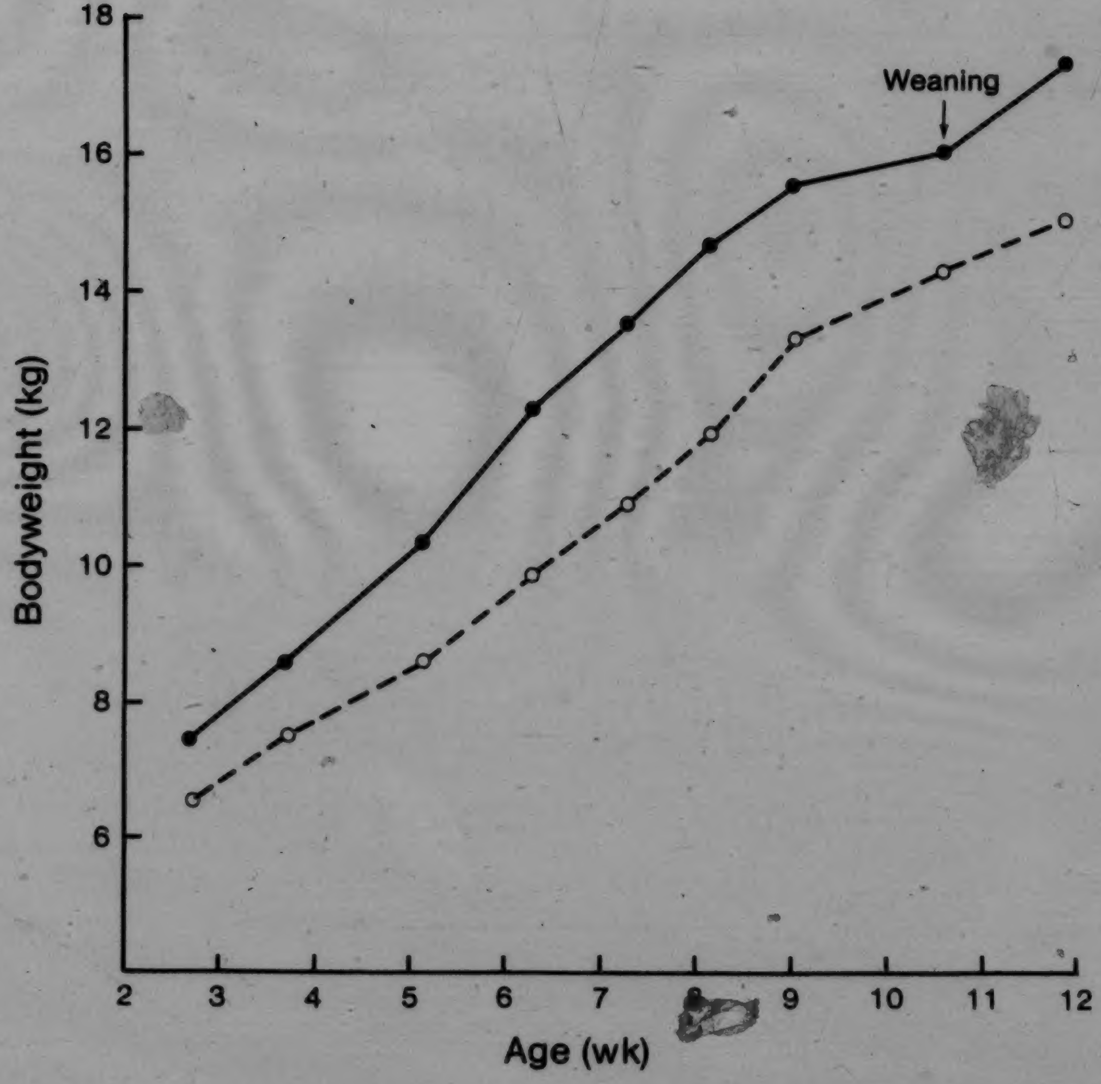


Table IV.4 Lamb Dimensions at 10 Weeks of Age, Experiment II[1]

Measurement:	CON (5)[2]	CE (5)
Bodyweight (kg)	16.0±1.11	14.3±1.14
Wither Height (cm)	49.8±1.41	47.6±1.95
Body Length[3] (cm)	50.0±1.17	46.3±1.76
Elbow-Knee Length (cm)	15.6±0.79	15.0±0.60
Knee-Hoof Length (cm)	16.1±0.57	14.6±0.70
Stifle-Hock Length (cm)	21.0±1.26	21.4±0.85
Hock-Hoof Length (cm)	23.1±0.49	19.6±0.70**
Head Length (cm)	15.6±0.44	14.6±0.32†
Ear Length (cm)	10.5±0.54	8.4±0.32**
Ear Width (cm)	6.5±0.15	5.0±0.24‡
Ear Area[4] (cm^2)	55.5±3.60	34.3±2.51‡

[1] values represent mean±SEM.

[2] number of animals/treatment in parentheses.

[3] body length measured from the point of shoulder to the point of buttock.

[4] ear area calculated as the area of an ellipse.

† treatment means are significantly different (P<0.10).

**treatment means are significantly different (P<0.01).

‡ treatment means are significantly different (P<0.001).

Table IV.5 Leg Bone Lengths of the 13 Week Old Lambs, Experiment II[1]

Measurement	CON (4)[2]	CE (5)
Bodyweight (kg)	17.8±1.59	14.8±1.55
Foreleg:		
Scapula (cm)	10.5±0.09	10.3±0.08
Humerus (cm)	12.2±0.32	11.5±0.40
Radius[3] (cm)	11.8±0.21	11.2±0.19
Metacarpus[3] (cm)	11.0±0.28	9.8±0.25
Phalanx (cm)	3.1±0.11	2.8±0.13
Hindleg:		
Femur (cm)	14.3±0.35	13.5±0.50
Tibia (cm)	16.1±0.29	15.1±0.61
Metatarsus (cm)	11.8±0.35	11.2±0.81
Phalanx (cm)	3.2±0.10	3.1±0.13

[1] values represent mean±SEM.

[2] number of animals/treatment in parentheses.

[3] values adjusted for bodyweight as a covariate.

intakes (g/day) of the CE lambs were lower as a result of their slightly smaller bodyweights (Table IV.6). Cold exposure had no significant influence ($P>0.10$) on DM or N digestibility. Of the nitrogen that was digested however, 68 and 58 % for the CE and CON lambs, respectively, was excreted in the urine, resulting in a significantly decreased nitrogen retention (g/day, g/g digestible N intake) in the CE lambs ($P<0.05$).

Although the CON lambs averaged 17.8±0.16 kg while the CE animals weighed only 14.8±0.16 kg at the time of slaughter, the organ weights of the 13 wk old lambs, expressed on a per kg BW basis, revealed no significant treatment effect except for a tendency of increased abomasal and thyroid weights ($P<0.10$) as a result of cold exposure (Table IV.7).

Table IV.6 The Digestibility and Nitrogen Retention of a Concentrate Diet Fed to the Weaned Lambs, Experiment II[1]

Measurement:	CON (5)[2]	CE (5)
Bodyweight (kg)	17.3± 1.16	15.0±1.26
Intake (g/day)		
Dry Matter	426.04±29.48	380.78±30.24
Nitrogen	10.42± 0.72	8.95±0.55
Digestibility (%)		
Dry Matter	82.27± 0.65	82.79±0.44
Nitrogen	72.94± 0.89	73.18±1.20
Urinary N (g/day)	4.41± 0.36	4.46±0.28
Nitrogen Retention:		
g/day	3.21± 0.43	2.10±0.23*
mg.kg $BW^{-1}.day^{-1}$	184.34±18.96	139.08±7.79†
g/g DNI[3]	0.43± 0.04	0.32±0.02*

[1] values represent mean±SEM.

[2] number of animals/treatment in parentheses.

[3] DNI= digestible nitrogen intake.

† treatment means are significantly different (P<0.10).

* treatment means are significantly different (P<0.05).

Table IV.7 Organ Weights of the 13 Week Old Lambs, Experiment II[1]

Organ Weight (g/kg BW)	CON (5)[2]	CE (5)
Digestive Tract:		
Reticulum	2.87±0.16	3.48±0.27
Rumen	17.12±0.50	18.55±0.77
Omasum	1.65±0.13	1.76±0.13
Abomasum	5.03±0.45	6.73±0.65†
Duodenum	1.09±0.13	1.22±0.16
Jejunum & Ileum	29.32±2.06	32.54±2.26
Cecum	1.89±0.26	1.93±0.24
Colon	10.03±1.15	10.69±0.57
Heart	5.49±0.21	5.64±0.31
Lungs	11.31±0.60	11.64±0.35
Liver	22.15±1.14	22.17±0.80
Kidneys	3.61±0.18	3.71±0.20
Spleen	1.86±0.51	2.30±0.31
Testes[3]	1.44±0.06	1.40±0.26
Thymus	2.48±0.40	2.40±0.31
Endocrine Glands:		
Thyroid	0.053±0.002	0.065±0.005†
Adrenals	0.096±0.011	0.135±0.032

[1] values represent mean±SEM.

[2] number of animals/treatment in parentheses.

[3] values represent mean±SEM of 3 animals/treatment.

† treatment means are significantly different (P<0.10).

E. Discussion

Growth Rate

In the present experiments growth rate, as measured by body weight change, tended to be depressed in the lambs housed at 0 °C both prior to and following weaning. Since weight gain after weaning was assessed only over a 11 day period, it is doubtful if this measure is a reliable index of growth.

With milk-fed lambs fed at an equivalent level of intake, Gibb and Penning (1972) reported a reduction of ADG, measured from 1-23 days of age, of approximately 9 % if the lambs had been housed at 7 °C rather than 21 °C. No detrimental effect of temperature on ADG however was observed if the milk replacer was available ad-libitum (Gibb and Penning 1972). In contrast, Williams and Innes (1982) found a significant depression in ADG in calves kept for 2 wk at 3 °C and fed milk replacer ad-libitum. A cold-induced depression of variable magnitude in growth rate following weaning has also been observed in young pigs fed restricted amounts (Fuller and Boyne 1971; Hacker et al. 1973; Phillips et al. 1982), ad-lib fed lambs (Ames and Brink 1977) and feedlot steers (Brink and Schalles 1975) although the results obtained with ad-lib fed young pigs are inconsistent (Fuller 1965; Brown et al. 1976; Phillips et al. 1982).

A large portion of the variability in the literature concerning the occurence and magnitude of a cold-induced growth depression can be related to differences in the severity and/or duration of the cold stress imposed. Since, in the present studies, heat production was not measured and the lower critical temperature has not been established for this age of lamb, the degree of cold stress imposed by housing young lambs at 0 °C can only be approximated. Based on the thermoneutral heat production of 1-4 wk old lambs (Degen and Young 1982), published values of the lower critical temperature of very young lambs (Alexander 1961) and the thermal insulation of sheep and of other animals (Blaxter 1976; Christopherson and Young 1981), it would appear that a 4 wk old unshorn lamb would not be cold stressed at effective ambient temperatures above -1 °C. For the majority of time during the experimental period then it is possible that both groups of lambs were housed within their thermoneutral zone. The lack of a significant reduction in the growth rate of the CE lambs therefore can be related to the degree of cold stress imposed and the equivalent nutrient intakes of both groups of lambs prior to (Chapter II) and following weaning.

Lamb Development

Maintenance of thermal equilibrium in a cold environment might favor the differential development of young growing animals in comparison to their counterparts in a warm environment. This phenonomen has been clearly shown to occur in pigs by Weaver and Ingram (1969) who assigned littermates of weaned pigs to exposure temperatures of 35 °C or 5 °C and offered feed at a level so that weight gain was similar in both environments. They were able to demonstrate that after 72 days, the cold-exposed pigs were shorter and stockier, and had smaller extremity surface areas, as indicated by tail length, ear area and head length, than their littermates kept in a thermoneutral environment. Bone lengths measured after the animals were killed revealed shorter femur, tibia, metatarsal, humerus, radius, ulna and metacarpal lengths in the cold-stressed pigs (Weaver and Ingram 1969). Similarly, Heath and Ingram (1979) found that pigs raised at 25 °C had rounder bodies, shorter snouts and smaller extremities compared to their littermates housed at 35 °C. Hacker et al. (1973) also found that ear area, body and leg length and heart girth, adjusted for bodyweight, tended to be reduced in pigs housed at 2 °C rather than at 20 °C. The underlying control mechanisms involved in these morphological adaptations have yet to be identified but possibly could be related to a decreased tissue temperature and/or restricted blood flow to the extremity areas.

Similar trends of altered development were found in the present studies with young lambs, especially in the more distal leg bones and in the extremity surface areas. A greater severity and/or duration of cold exposure however may be necessary to induce a more pronounced change in the development of the lamb. These morphological changes in young lambs and in other animals might be expected to be beneficial to the thermal balance of the animals while living in a cold environment.

Digestibility-Nitrogen Balance Trial

Previous studies performed with mature sheep and cattle exposed to a cold environment over a prolonged period have indicated that DM digestibility of a roughage-based feed is depressed by approximately 0.1-0.3 digestibility units/°C (see NRC 1981). A cold-induced reduction in roughage digestibility has been found to be independent of the level of intake (Christopherson 1976) and is probably associated with an increase in rumination activity (Gonyou et al. 1979), reticulo-rumen motility (Westra and Christopherson 1976; Gonyou et al. 1979), rate of passage of digesta (Westra and Christopherson 1976; Kennedy et al. 1976,1977,1982) and change in site of digestion (Kennedy et al. 1976,1982). It is hypothesized however that the effect of cold exposure on digestibility may be diet-dependent; recent research has found that the digestibilities of a 50 % barley-50 % pelleted alfalfa (Young and Degen 1981) and a 100 % concentrate (Kennedy et al. 1982) diet fed to mature sheep were not affected by a cold environment. The lack of effect of cold exposure on the DM digestibility of the 100 % concentrate ration fed to the lambs in the present study is consistent with this hypothesis.

Conflicting evidence exists in the literature as to the influence of a low ambient temperature on N digestibility. In ruminants fed a roughage-based diet, N digestibility has been reported to be reduced (Christopherson and Milligan 1973; Christopherson and Thompson 1973; Ames and Brink 1977;

Kennedy and Milligan 1978; Kennedy et al. 1982) or unchanged (Kennedy et al. 1976; Westra and Christopherson 1976) as a result of a low temperature. The discrepancies in reported results do not appear to be related to treatment differences in the level of feed intake. N digestibility of a 50 % concentrate diet fed to sheep and cattle has also been found to be reduced during cold exposure (Christopherson 1976) although N digestibility in mature sheep fed a 100 % concentrate ration was not affected by a low ambient temperature (Kennedy et al. 1982), nor was N digestibility depressed in the CE lambs in the present experiment.

Despite similar N digestibilities for the CON and CE lambs, N retention was reduced during cold exposure as a result of increased urinary N excretion (% digestible N intake). The decreased N retention is consistent with the reduced body weight gain of the lambs in the cold. Similarly, reductions in N retention have been reported in cold exposed ruminants fed either ad-libitum or restricted to the same level as control animals, kept at a thermoneutral temperature (Christopherson and Milligan 1973; Westra and Christopherson 1976; Ames and Brink 1977; Kennedy and Milligan 1978).

Organ Weights

Digestive Organs

Exposure to a cold environment was accompanied by a slight increase in weight relative to total body weight of each component of the gastrointestinal tract;

however, only the increase in abomasal weight approached significance ($P<0.10$). Although it is known that digestive functions often are affected by cold exposure (NRC 1981), other studies performed with growing pigs housed at a low ambient temperature have failed to reveal any cold-induced change in gastrointestinal tract weight (Sugahara et al. 1970; Hacker et al. 1973; Brown et al. 1976). Digestive organ weights of lactating ewes also have not been found to be affected by a prolonged period of cold exposure (Chapter II). In contrast, wethers chronically exposed to low temperatures have been shown to have increased small intestine (Schaefer 1979), reticulo-rumen and total gastrointestinal tract (Graham et al. 1982) weights, although these effects were likely a consequence of an increased feed intake rather than the cold environment per se.

Other Organs and Glands

Treatment means for heart, liver, kidney, spleen and lung weights also tended to be higher for the cold-exposed lambs although the differences between treatment groups were not significant. A significantly increased heart weight in cold-stressed animals has been found for wethers (Schaefer 1979) and growing pigs (Fuller 1965), however other studies have failed to show any temperature-heart weight interaction (Hacker et al. 1973; Brown et al. 1976; Graham et al. 1982). Assuming that an increase in heart weight is a reflection of a greater work load, an increased heart weight would only occur if the severity of cold stress was sufficient to result in an increased cardiac output and/or arterial pressure.

Since liver metabolism has been shown to be affected by cold exposure (Thompson et al. 1975), weight changes in this organ might be expected. A significant increase in liver weight has been reported for cold-stressed wethers (Schaefer 1979; Graham et al. 1982) and pigs (Sugahara 1970); however, this effect again may be related to a higher level of feed intake rather than to the low ambient temperature. In growing pigs fed a restricted, but equivalent level of intake, liver weight was depressed in the cold-exposed animals in comparison to their counterparts housed at a thermoneutral temperature (Hacker et al. 1973).

Greater urinary excretion and/or food intake during cold exposure may result in an increased kidney weight. Larger kidney weights have been found in cold-stressed wethers (Schaefer 1979) and growing pigs (Fuller 1965; Sugahara 1970).

A larger spleen size has been reported by Fuller (1965) and Brown et al. (1976) in cold-stressed pigs, perhaps reflecting an increased blood storage capacity, haemotopoiesis or erythropoietin secretion (Swenson 1970) during cold exposure.

From a survey of the present literature, a cold-induced change in lung weight has not been established in any study.

The lower testicle weight of the CE lambs in the present study is consistent with the results of Hacker et al. (1973) obtained with young pigs. A reduction in external gonad weight again may be of benefit to the thermal equilibrium of the animal but may also reflect a retardation of sexual development, as evidenced by the cold-induced endocrinological alterations in boars reported by Hacker et al. (1973).

A tendency towards increased thyroid and adrenal gland weights in the CE lambs also agrees well with the reported cold-induced changes in circulating levels of thyroid and adrenal hormones in domestic ruminants (Westra and Christopherson 1976; Thompson et al. 1978; Christopherson et al. 1979; Graham et al. 1981).

Lamb organ weight relative to body weight was found to be similar in most cases to that found previously in this laboratory with mature sheep; noteable exceptions being the smaller lamb omasal, thyroid gland and spleen weights but greater weight of the small intestine (Table IV.8).

In summary, the exposure of young lambs to a cold environment appeared to result in certain alterations in development although the duration of cold stress perhaps was insufficient to demonstrate the full extent of their

Table IV.8 Organ Weights of Sheep Exposed to Thermoneutral and Chronically Cold Environments

	Thermoneutral				Cold			
Experiment	Present Study	Chapter II	Graham et al. 1982	Schaefer 1979	Present Study	Chapter II	Graham et al. 1982	Schaefer 1979
Animals	Lambs	Lactating Ewes	Wethers	Wethers	Lambs	Lactating Ewes	Wethers	Wethers
Exposure Temperature (°C)	21	21	19-24	18	0	0	8-13	3
Duration of Exposure (wk)	11	9	8	10-12	11	9	8	10-12
Organ Weight (g/kg BW)								
Digestive Tract:								
Reticulo-rumen	20.0	27.9	20.6	18.2	22.0	28.8	22.0	17.4
Omasum	1.7	3.5		3.3	1.8	2.8		3.1
Abomasum	5.0	6.9	6.4	5.6	6.7	6.8	5.3	6.7
Small Intestine	30.4	17.7	14.0	16.2	33.8	17.3	15.8	22.5
Large Intestine	11.9	17.2	13.2	13.7	12.6	15.7	13.2	12.3
Other Organs:								
Heart	5.5		4.8	4.5	5.6		4.2	5.7
Lungs	11.3	11.7		14.6	11.6	11.4		11.3
Liver	22.2		14.8	16.3	22.2		16.5	24.0
Kidneys	3.6	3.5		3.2	3.7	3.2		3.8
Spleen	1.9			3.6	2.3			3.0
Endocrine Glands:								
Thyroid	0.05	0.11		0.16	0.07	0.13		0.18
Adrenals	0.10	0.12		0.20	0.14	0.15		0.19

biological responses.

The pattern of growth, N excretion and N retention of the cold-exposed lambs following weaning probably reflects the increased use of amino acids as energy substrates. Further work is required to determine if the provision of additional food energy to the cold exposed lambs might have prevented the increased utilization of protein as an energy source and spared it for use in growth.

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V. General Summary and Conclusions

The most significant aspects of these experiments can be summarized as follows:

1. Milk production and milk energy output in ewes suckling single lambs were not altered by chronic cold exposure. Since cold exposure resulted in a reduced feed digestibility and increased heat production without any apparent loss of bodyweight, it can not be concluded by what means milk production was maintained. Possibly the methods used to estimate milk production gave erroneous results or else heat production estimates of the cold-exposed lactating ewes were overestimated because of measurement-induced changes of behavior. However, the calculations of net energy balance suggested that energy substrates may have been mobilized from body tissues to a greater extent in the CE ewes, resulting in changes in body tissue composition.
2. Chronic cold exposure resulted in a change in mammary fat metabolism as indicated by milk fat concentration and fatty acid composition. Milk fat composition changes were indicative of an increased supply of long chain fatty acids, likely mobilized from adipose tissue.
3. Endocrinological changes in the cold-exposed lactating ewes, as indicated by plasma levels of hormones, were similar to changes previously reported for cold-stressed mature wethers. High levels of catecholamines and T_3 relative to insulin would be expected to promote an

increased mobilization of body energy reserves to help support an increased level of thermogenesis and to maintain milk secretion during cold exposure.

4. A reduction of mammary weight as a consequence of chronic cold exposure was observed. Theoretically this would suggest that milk production would be impaired, although measures of milk yield and mammary efficiency would indicate the contrary. Further work is needed to investigate this area.
5. Both chronic and acute cold exposure appeared to decrease mammary blood flow. From the examination of the diurnal variation in mammary blood flow it would appear that the influence of temperature is highly dependent upon the animal's posture. Changes in behavior during cold exposure therefore could minimize any influence of temperature on blood flow to the mammary gland.
6. Cold exposure appeared to result in a redistribution of blood flow within the mammary gland. This may be related to a general mechanism of an elevated adipose tissue blood flow to increase free fatty acid mobilization during cold exposure.
7. Suckling appeared to act, as did minor stress, to reduce mammary blood flow. While the action of stress on blood flow may be related to the release of adrenaline and noradrenaline, the mechanism and function of a suckling-induced reduction of mammary blood flow remains to be defined.

8. While nutrient intake prior to weaning was determined to be similar for both the control and cold-exposed lambs, only a slight depression in growth rate as a result of cold exposure was observed. Possibly the thermal stress imposed was not sufficient to result in an increased metabolic rate and maintenance energy demand of the young lambs.
9. DM and N digestibilities of a concentrate diet fed to the young lambs were not affected by cold exposure although a relative increase in urinary N loss resulted in a decreased N retention in the cold-exposed lambs. These results would tend to support the reduced rate of growth of the cold-exposed lambs observed over a short period following weaning.
10. Morphological adaptations to the cold seemed to be developing by 13 wk of age especially in terms of the distal leg bones and ear surface area. The benefits of these adaptations as related to a decreased rate of heat loss to the environment remain to be quantitatively examined.
11. Prolonged cold exposure did not have any effect on gastro-intestinal tract weight in either the lactating ewe or young lamb, suggesting that digestive responses that occur as a result of cold exposure are independent of gross changes in digestive tract morphology.